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AssayWise Letters

HRP (Horseradish Peroxidase) Detection

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Advancing Assay & Test Technologies

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HRP (Horseradish Peroxidase) Detection

The enzyme horseradish peroxidase (HRP), found in the roots of horseradish, is used extensively in biochemistry applications primarily for its ability to amplify a weak signal and increase detectability of a target molecule. HRP is often used in conjugates (molecules that have been joined genetically or chemically) to determine the presence of a molecular target. HRP is also commonly used in techniques such as ELISA and immunohistochemistry due to its monomeric nature and the ease with which it produces colored products. Peroxidase, a heme-containing oxidoreductase, is a commercially important enzyme which catalyzes the reductive cleavage of hydrogen peroxide by an electron donor.

Horseradish peroxidase is ideal in many respects for these applications because it is smaller, more stable, and less expensive than other popular alternatives such as alkaline phosphatase. It also has a high turnover rate that allows generation of strong signals in a relatively short time span. It should be noted that high concentrations of phosphate severely decrease stability of horseradish peroxidase. In addition to biomedical applications, horseradish peroxidase is one of the enzymes with important environmental applications. This enzyme is suitable for the removal of hydroxylated aromatic compounds (HACs) that are considered to be primary pollutants in a wide variety of industrial wastewater.

ReadiUse™ Colorimetric HRP Substrate Solutions

HRP and HRP conjugates facilitate the ABTS oxidation in the presence of hydrogen peroxide, turning ABTS into its blue-green oxidized product. This chromogenic reaction is widely used to quantify HRP in ELISA assays. The oxidized ABTS product has the absorption maximum at 420 nm that can easily be followed with a spectrophotometer. ReadiUse™ ABTS Substrate Solution (Cat# 11001) is optimized for ELISA assays that use HRP or HRP-labeled conjugates and hydrogen peroxide in microwell plates or test tubes. Our ABTS solution allows HRP reaction to be done with a single addition. The assay solution changes its color to light green upon its reaction with HRP or HRP conjugates.

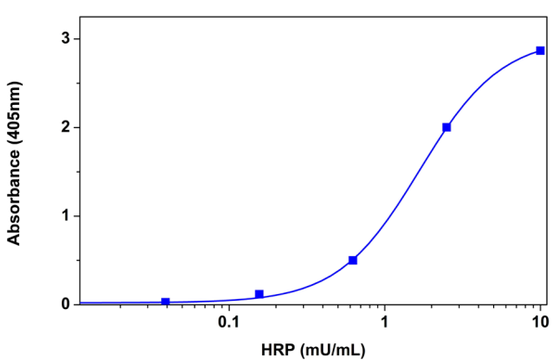

Figure 1. HRP dose responses were measured with ReadiUse™ ABTS Substrate Solution (Cat#11001) in a 96-well clear plate using a SpectraMax® microplate reader (Molecular Devices).

ReadiUse™ TMB Substrate Solution (Cat# 11003) is a premixed solution of TMB substrate with hydrogen peroxide. It produces a blue product upon interaction with HRP or HRP conjugates without the addition of hydrogen peroxide. The soluble blue product can be quantitated at 650 nm. The use of a stop solution produces 2-4 fold increase in sensitivity and the resulting yellow solution can be read at 450 nm. ReadiUse™ TMB Substrate Solution provides a convenient and ultrasensitive quantitative substrate system.

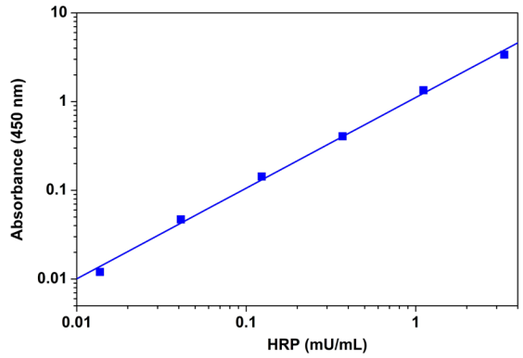

Figure 2. HRP dose responses were measured with ReadiUse™ TMB Substrate Solution (Cat# 11003) in a 96-well clear plate using a SpectraMax® microplate reader (Molecular Devices). As low as 3 µU/well peroxidase was detected with 10 minutes incubation.

ReadiUse™ Hydrogen Peroxide Solution

Compared with other commercial hydrogen peroxide solutions, our formulated ReadiUse™ Hydrogen Peroxide Solution (Cat# 11004) is much more stable. It is calibrated to ensure more reproducible peroxidase-based assays.

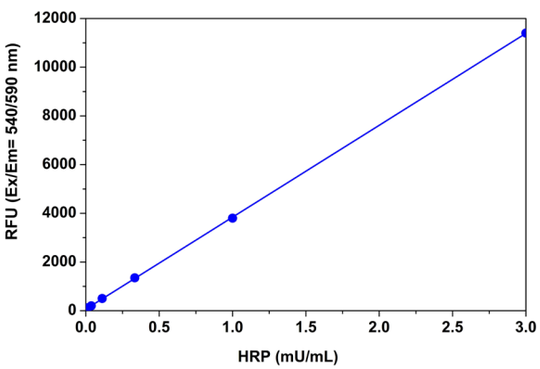

Figure 3. HRP dose responses on a 384-well plate were measured with Amplite™ ADHP (Cat# 11000) and ReadiUse™ Hydrogen Peroxide Solution (Cat# 11004).

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Fluorimetric HRP Detection Substrates

A variety of substrates are available for detecting peroxidase activity in ELISA assays. Colorimetric HRP substrates (e.g., TMB, OPD and ABTS) have been widely used for years. Each of these substrates varies greatly with its performance characteristics such as detection sensitivity, working range and attainable signal-to-noise ratio. Amplite™ Blue (Cat# 11005) is a soluble fluorogenic HRP substrate for the detection of peroxidase activity. Amplite™ Blue allows rapid HRP detection assays to be performed with greater sensitivity than the colorimetric HRP substrates. The fluorescent product of Amplite™ Blue does not photobleach. Amplite™ Blue exhibits a flat baseline in assays, which facilitates low-level detection sensitivity and allows for high signal-to-noise ratio.

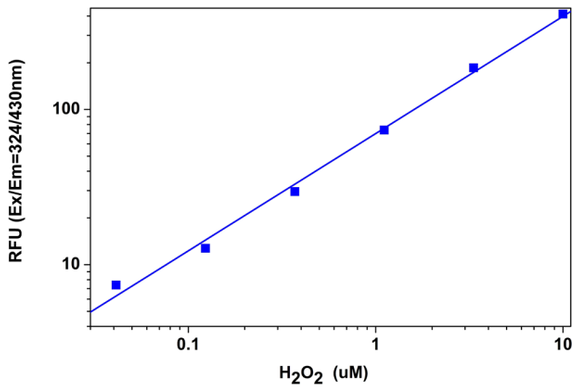

Figure 4. H_2O_2 dose responses measured in a solid black 96-well plate with Amplite™ Blue (Cat# 11005).

Amplite™ IR (Cat# 11009) is a fluorogenic peroxidase substrate that generates near infrared fluorescence upon reaction with peroxidase and H_2O_2. It can be used to detect both H_2O_2 and peroxidase. Amplite™ IR generates a substance that has maximum absorption at 647 nm with maximum emission at 670 nm. This near infrared absorption and fluorescence minimize the assay background often caused by the autoabsorption and/or autofluorescence of biological samples that rarely absorb light beyond 600 nm. Unlike other HRP substrates, such as dihydrofluoresceins and dihydrorhodamines, the air-oxidation of Amplite™ IR is minimal. Compared with ADHP (also called Amplex® Red in literature), Amplite™ IR generates the fluorescence that is pH-independent from pH 4 to 10. Thus it is a superior alternative to ADHP (Amplex® Red) for detections that require low pH where ADHP (Amplex® Red) has reduced fluorescence. We have used Amplite™ IR to detect HRP in quite a few immunoassays. Amplite™ IR can also be used to detect a trace amount of H_2O_2. Because H_2O_2 is produced in many enzymatic redox reactions, Amplite™ IR can be used in coupled enzymatic reactions to detect the activity of many oxidases and/or related enzymes/substrates or cofactors such as glucose, acetylcholine, cholesterol, L-glutamate, and amino acids, etc.

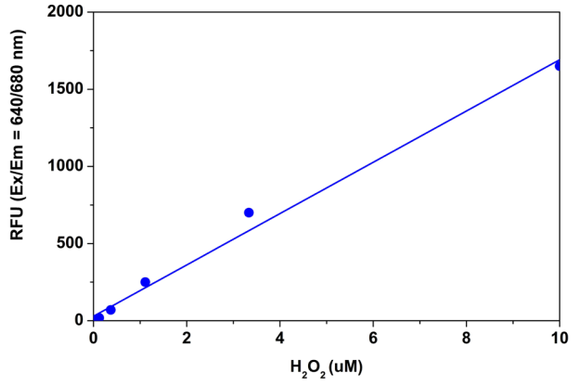

Figure 5. H_2O_2 dose responses measured in a solid black 96-well plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11502). As low as 0.03 μM H_2O_2 was detected.

Amplite™ ADHP (Cat# 11000) is chemically similar to Amplex® Red (Amplex® Red is the trademark of Invitrogen). It is a sensitive fluorogenic peroxidase substrate. Amplite™ ADHP has the highest quality of ADHP with much lower background than the materials from other commercial vendors. Amplite™ ADHP generates highly fluorescent resorufin that has maximum absorption at 571 nm and maximum emission at 585 nm. Unlike other HRP substrates, such as dihydrofluoresceins and dihydrorhodamines, the air-oxidation of Amplite™ ADHP is minimal. So far ADHP has been known as the most sensitive and stable fluorogenic probe for detecting HRP and H_2O_2. ADHP has been widely used to detect HRP in many immunoassays. On the other hand, ADHP can also be used to detect a trace amount of H_2O_2. The ADHP-based H_2O_2 detection is at least

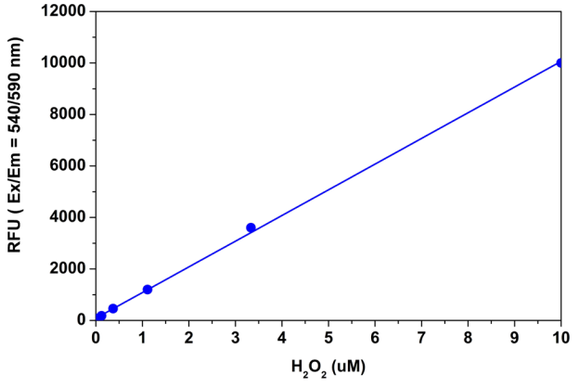

Figure 6. H_2O_2 dose responses measured in a solid blakc 384-well plate with Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit (Cat# 11501). As low as 0.03 μM H_2O_2 was detected.

one Because H_2O_2 is produced in many enzymatic redox reactions, ADHP can be used in coupled enzymatic reactions to detect the activity of many oxidases and/or related enzymes/substrates or cofactors such as glucose, acetylcholine, cholesterol, L-glutamate and amino acids, etc.

HRP Reaction Stopping Solution

HRP coupling reactions provide sensitive biomolecular assays based on hydrogen peroxide–generating enzyme systems linked to peroxidase–mediated oxidation. Fluorogenic HRP substrates are preferred for enhancing assay sensitivity. Among them, the most commonly used HRP substrates include ADHP (also called Amplex® Red), Amplex® UltraRed and Amplite™ Red. Typically, detection reactions are performed in microplate wells and are initiated by adding a fluorogenic HRP substrate, resulting in continuous fluorescence increase. It is critical to ensure that the timing of the standard and unknown sample measurements is the same. Signal Guard™ HRP Reaction Stopping Solution (Cat# 11020) provides convenience and control by allowing the fluorescence signal-generating reaction to be terminated at a user-determined time point. After the addition of the stop reagent, the fluorescence signal remains stable. The Signal Guard™ HRP Reaction Stopping Solution is designed to be used in conjunction with ADHP (Amplex® Red), Amplite™ Red and Amplex® UltraRed fluorogenic substrates. It can also be used in other HRP reaction systems.

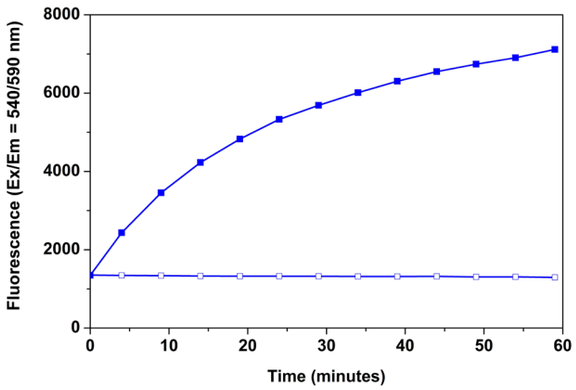

Figure 7. Aplication of Signal Guard™ HRP Reaction Stopping Solution (Cat# 11020) on HRP coupled glucose detection reaction. Reactions were incubated at room temperature for 5 minutes and then 20 µL 1X stop reagent was added, and the reaction was completely inhibited by Signal Guard™ HRP Reaction Stopping Solution.

HRP Conjugation Tools

ReadiUse™ Preactivated HRP NHS Ester (Cat# 11025) is the mono NHS ester of HRP. It can be used to readily label proteins (such as antibodies) or other biological molecules that have an amino group. ReadiUse™ Preactivated HRP NHS Ester is robust and easy to use. It can used to label antibodies with a simple mixing, i.e. the basic solution of an antibody (pH 8.5-9.0) can be directly mixed with the HRP NHS ester and shaken for 1-2 hours. In most cases, the resulting solution can be directly used for ELISA assays without further purification.

ReadiUse™ Preactivated HRP Maleimide (Cat# 11026) is a thiol-reactive HRP derivative. It can be used to readily label proteins (such as antibodies) or other biological molecules that have a thiol group. ReadiUse™ Preactivated HRP Maleimide is robust and easy to use. It can be used to label antibodies with a simple mixing, i.e. the slightly acidic or neutral solution of an antibody (pH 6-7) can be directly mixed with the HRP maleimide, and the reaction mixture is shaken for 1-2 hours. In most cases, the resulting solution can be directly used for ELISA assays without further purification.

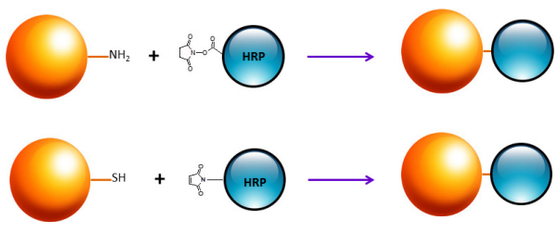

Figure 8. The labeling principles of HRP NHS ester (top) and HRP maleimide (bottom) with proteins and other biomolecules.

Protein-protein conjugations are commonly performed with a bifunctional linker (such as the commonly used SMCC), which has different reactivity on each end for linking two different proteins. One end of the crosslinker reacts (via NHS ester) with amines (-NH_2) found in the amino acid lysine and N-terminus, and the other end reacts (via maleimide) with the thiol groups (-SH) found in the amino acid cysteine. SMCC-modified proteins are extremely unstable and often self-reactive since proteins often contain both amine and thiol groups that cause a significant amount of homo-crosslinking. In addition, it is quite difficult and tedious to quantify the number of maleimide groups on a protein. ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kits (Cat# 5503 & 5504) are designed for preparing horseradish peroxidase (HRP) conjugates directly from proteins, peptides, and other ligands that contain a free amino group. The HRP provided in our kits has been pre-activated with our proprietary linker Buccutite™ FOL, and can be directly used for conjugation. The Buccutite™ FOL-activated HRP readily reacts with Buccutite™ MTA-containing molecules under mild neutral conditions without any catalyst required. Compared with commonly used SMCC and other similar technologies, our Buccutite™ bioconjugation system is much more robust and easier to use. It enables faster and quantitative conjugation of biomolecules with higher efficiencies and yields. Kit 5503 is optimized for

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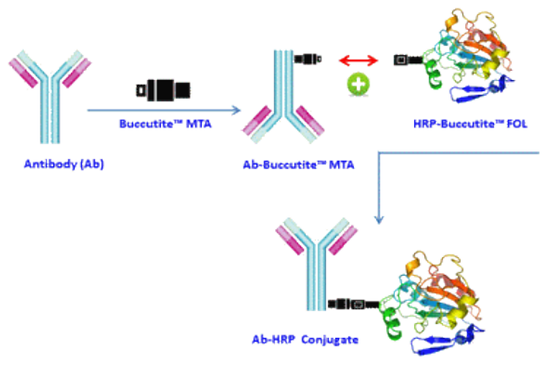

Figure 9. The working principle of ReadiLink™ HRP Antibody Conjugation Kits (Cat# 5503 & 5504).

labeling 100 µg protein, and Kit 5504 is optimized for 1 mg protein.

The major advantage of a liquid HRP-conjugate is that it eliminates the chance for human error when dissolving or diluting the lyophilized or concentrated conjugate. Adding too much or not enough buffer results in assay to assay variation. Until now, the disadvantage of this format is the inherent instability of the liquid HRP-conjugates. Signal Guard™ HRP Conjugate Stabilizer (Cat# 11010) is formulated to allow you to provide pre-diluted, ready-to-use conjugates. This formulation can be used to create stock solutions (1:1,000) or ready-to-use assay reagents (1:100,000) that can be stored at 4°C for 24 months or at room temperature for six months.

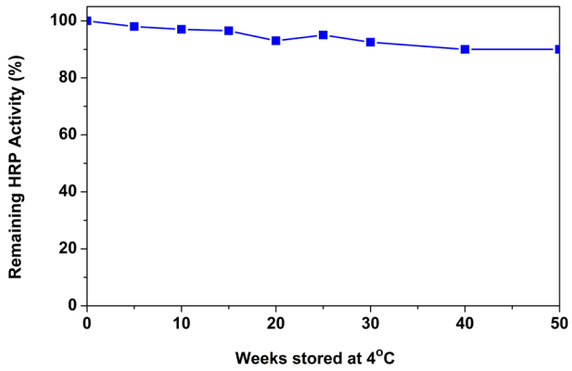

Figure 10. Goat Anti-Rabbit conjugate with Signal Guard™ HRP Conjugate Stabilizer (Cat# 11010) was stored at 4 ºC and tested over 50 weeks using Amplite™ ADHP (Cat# 11000).

Table 1. HRP (Horseradish Peroxidase) Detection Probes and Assays

Cat #	Product Name	Size	Ex (nm)	Em (nm)
11000	Amplite™ ADHP [Equivalent to Amplex® Red] *CAS#: 119171-73-2*	25 mg	571	585
11005	Amplite™ Blue	25 mg	324	409
11502	Amplite™ Fluorimetric Hydrogen Peroxide Assay Kit *Near Infrared Fluorescence*	500 tests	647	670
11009	Amplite™ IR	1 mg	647	670
11011	Amplite™ Red	1000 assays	571	585
5503	ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 100 µg Protein*	1 kit	N/A	N/A
5504	ReadiLink™ Peroxidase (HRP) Antibody Conjugation Kit *Optimized for Labeling 1 mg Protein*	1 kit	N/A	N/A
11001	ReadiUse™ ABTS Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L	420	N/A
11004	ReadiUse™ Hydrogen Peroxide Solution *50 mM Calibrated and Stabilized Solution*	5x10 mL	N/A	N/A
11026	ReadiUse™ Preactivated HRP Maleimide	100 µg	N/A	N/A
11025	ReadiUse™ Preactivated HRP NHS Ester	100 µg	N/A	N/A
11003	ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	1 L	650	N/A
11012	ReadiUse™ TMB Substrate Solution *Optimized for ELISA Assays with HRP Conjugates*	100 mL	650	N/A
11010	Signal Guard™ HRP Conjugate Stabilizer	50 mL	N/A	N/A
11020	Signal Guard™ HRP Reaction Stopping Solution	0.5 mL	N/A	N/A

ReadiLink™ Antibody Labeling Technology

ReadiLink™ iFluor™ Antibody Labeling Kits provide a convenient way to label antibodies using a stable reactive form of the iFluor™ dyes. The iFluor™ dyes show good reactivity and selectivity with the aliphatic amines of antibodies and forms a carboxamide bond, which is identical to, and is as stable as the natural peptide bond. iFluor™-antibody conjugates may be used for immunofluorescent staining, fluorescent *in situ* hybridization, flow cytometry and other biological applications. Each kit comes with all the essential components for performing the conjugation reaction and for purifying the iFluor™-antibody conjugates. ReadiLink™ Kits quickly label your antibody of interest in two easy steps with the unique chemistry of ReadiLink™ Dyes. The conjugation kits provide the best method for readily labeling microscale volumes without requiring column purification.

Key Features of ReadiLink™ Kits :

- Save time with no purification requried.
- Conjugate your antibodies in 2 easy steps.
- Available in 10 different fluorophores with excitation wavelengths ranging from UV to infrared.

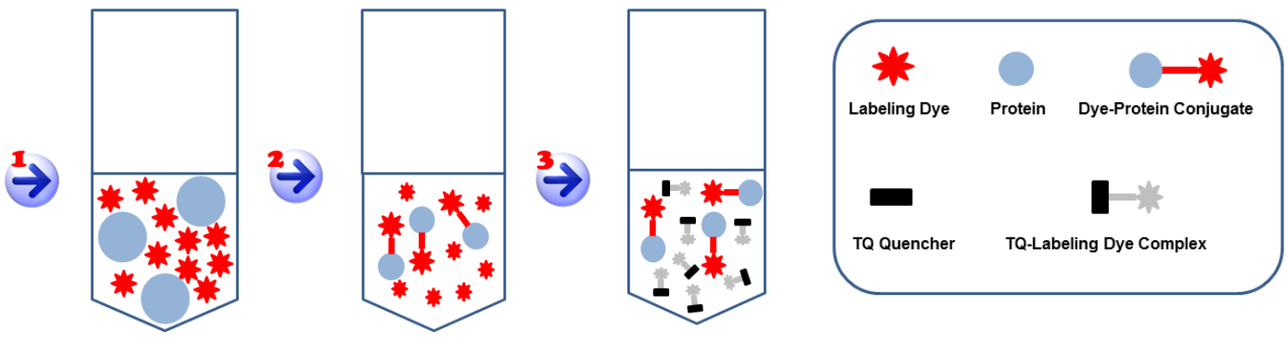

Figure 11. ReadiLink™ Kit Labeling Principle

- **Step 1.** Start the labeling reaction by mixing a labeling dye with a protein (to be labeled) in the reaction buffer (pH 7.5-8.5).
- **Step 2.** Incubate the reaction solution and get a mixture of the desired protein conjugate and unreactive free dye.
- **Step 3.** Quench the reaction by mixing a non-fluorescent Tide Quencher™ (TQ) dye with the reaction solution. The TQ dye stops the reaction AND converts the unreactive free labeling dye to the non-fluorescent TQ-labeled dye complex, which eliminates the background fluorescence interference of the free labeling dye.

Table 2. ReadiLink™ iFluor™ Antibody Labeling Assays

Cat #	Product Name	Alternative to	Size	Ex (nm)	Em (nm)
1299	ReadiLink™ FITC Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	492	516
1220	ReadiLink™ Rapid iFluor™ 350 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	345	442
1255	ReadiLink™ Rapid iFluor™ 488 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	491	514
1227	ReadiLink™ Rapid iFluor™ 555 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	559	569
1230	ReadiLink™ Rapid iFluor™ 594 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	592	614
1260	ReadiLink™ Rapid iFluor™ 633 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	638	655
1235	ReadiLink™ Rapid iFluor™ 647 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	654	674
1240	ReadiLink™ Rapid iFluor™ 680 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	682	701
1245	ReadiLink™ Rapid iFluor™ 700 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	693	713
1250	ReadiLink™ Rapid iFluor™ 750 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	753	779
1265	ReadiLink™ Rapid iFluor™ 790 Antibody Labeling Kit	Lightning-Link® Dye Labeling Kits	2 labelings	782	811

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ReadiView™ Biotinylation Technology

Biotin is a vitamin (Vitamin H, Vitamin B7, Coenzyme R) that is present in small amounts in all living cells and is critical for a number of biological processes including cell growth and the citric acid cycle. Biotin is abundant in certain plant and animal tissues such as corn kernels, egg yolk, brain, liver and blood. The valeric acid side chain of the biotin molecule can be derivatized in order to incorporate various reactive groups that facilitate the addition of a biotin tag to other molecules. Because biotin is relatively small (244.3 Daltons), it can be conjugated to many proteins and other molecules without significantly altering their biological activity. The highly specific interaction of biotin-binding proteins with biotin make it a useful tool in assay systems designed to detect and target biological analytes.

Once biotin is attached to a molecule, the biotin tag can be used to facilitate affinity purification of that molecule using an immobilized biotin-binding protein. Alternatively, a biotinylated molecule can be immobilized through interaction with a biotin-binding protein, and then used to affinity purify other molecules that specifically interact with it (i.e., co-immunoprecipitation or pull-down assays). In the context of immunohistochemistry and immunoblotting, biotin is most often conjugated to primary or secondary antibodies, and the biotin tag is then detected with a biotin-binding protein that is conjugated to an enzyme, fluorophore or other reporter molecule. Many proteins, such as antibodies, can be labeled with several biotin tags, each able to be bound by a biotin-binding protein. An optimized biotin-to-probe ratio can greatly increase the signal output of a detection system making it possible to create very sensitive assays.

Although a large number of biotin-labeled bioconjugates are commercially available, the accurate determination of biotinylation degree (ratio of biotin/biopolymer) is still a great challenge for biochemists. HABA is still predominantly used for determining the degree of biotinylation (through its absorption with the extinction coefficient = 34,000/$M^{-1}cm^{-1}$). When a biotin-containing sample is added, the biotin binds strongly to avidin and displaces the weakly bound HABA. The resulting decrease in absorbance relates to the amount of biotin. However, there are many factors that affect the accuracy of the HABA method, making it unreliable for many biotin-labeled conjugates.

ReadiView™ biotin contains a specially designed color tag (CT) that makes the biotinylation degree readily accessible by simply calculating the corrected absorption ratio of 280 nm/385 nm. The specially designed tag has very minimal effect on the biotin binding affinity, and its absorption maximum is designed to make the tag have minimal quenching effect on most fluorophores that are used for labeling avidins.

ReadiLink™ Protein Biotinylation Kit (Cat# 5521) is primarily used to prepare of biotin-labeled IgG for enzyme immunoassays (EIA). The kit uses our ReadiView™ Biotin Succinimidyl Ester (Cat# 3059) that reacts with an amino group of IgG and other biomolecules. Our unique biotin contained in the kit carries a color tag for indicating the degree of biotinylation, thus eliminating the need for the troublesome HABA biotinylation determination. The kit contains all of the necessary reagents for labeling and purification. Based on our in-house experiments, 5 to 8 biotin molecules can be conjugated to each IgG molecule using ReadiLink™ Protein Biotinylation Kit.

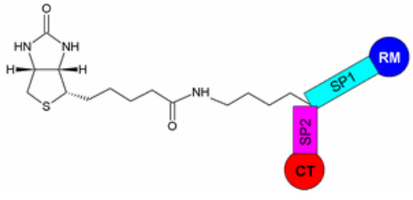

Figure 12. The chemical structure of ReadiView™ Biotin. RM = reactive moiety for labeling purpose; CT = color tag for calculating biotinylation degree.

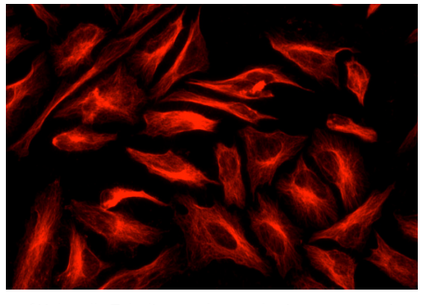

Figure 13. Image of Hela cells. Tubulin was detected using mouse anti-α-tubulin antibody staining, followed with ReadiView™ Biotin-Goat Anti-Mouse IgG conjugate staining, and then visualized with red-fluorescent streptavidin-ifluor™ 555.

Table 3. ReadiView™ Biotinylation Probes

Cat #	Product Name	Size
5521	ReadiLink™ Protein Biotinylation Kit *Powered by ReadiView™ Biotin Visionization Technology*	3 reactions
3050	ReadiView™ Biotin Acid	25 mg
3053	ReadiView™ Biotin Amine	5 mg
3055	ReadiView™ Biotin Hydrazide	5 mg
3058	ReadiView™ Biotin Maleimide	5 mg
3059	ReadiView™ Biotin Succinimidyl Ester	5 mg

TR-FRET Assays

Time-resolved fluorescence energy transfer (TR-FRET) is the practical combination of time-resolved fluorometry (TRF) combined with Förster resonance energy transfer (FRET) that offers a powerful tool for drug discovery researchers. TR-FRET combines the low background aspect of TRF with the homogeneous assay format of FRET. The resulting assay provides an increase in flexibility, reliability and sensitivity in addition to higher throughput and fewer false positive/false negative results. FRET involves two fluorophores, a donor and an acceptor. Excitation of the donor by an energy source (e.g. flash lamp or laser) produces an energy transfer to the acceptor if the two are within a given proximity to each other. The acceptor in turn emits light at its characteristic wavelength.

The FRET aspect of the technology is driven by several factors, including spectral overlap and the proximity of the fluorophores involved, wherein energy transfer occurs only when the distance between the donor and the acceptor is small enough. Through measurement of this energy transfer, interactions between biomolecules can be assessed by coupling each partner with a fluorescent label and detecting the level of energy transfer. Acceptor emission as a measure of energy transfer can be detected without needing to separate bound from unbound assay components (e.g. a filtration or wash step) resulting in reduced assay time and cost.

Homogeneous, mix-and-read TR-FRET assays offer advantages over other biomolecular screening assays, such as fluorescence polarization (FP) or TRF assays. In FP assays, background fluorescence due to library compounds is normally depolarized and background signal due to scattered light (e.g. precipitated compounds) is normally polarized. Depending on the assay configuration, either case can lead to a false positive or false negative result. However, because the donor species used in a TR-FRET assay has a fluorescent lifetime that is many orders of magnitude longer than background fluorescence or scattered light, emission signal resulting from energy transfer can be measured after any interfering signal has completely decayed. TR-FRET assays can also be formatted to use limiting receptor and excess tracer concentrations (unlike FP assays), which can provide further cost savings. In the case of TRF assays, a wash step is required to remove unbound fluorescent reagents prior to measuring the activity signal of the assay. This increases reagent use, time to complete the assay, and limits the ability to miniaturize the system (e.g. converting from a 384-well microtiter plate to a 1536-well plate). TR-FRET assays take advantage of the required proximity of the donor and acceptor species for generation of signal.

FRET No Wash cAMP Assay

Screen Quest™ FRET No Wash cAMP Assay Kit (Cat# 36379) provides a convenient assay method for monitoring the activation of adenylyl cyclase in G-protein coupled receptor systems. Compared with other commercial ELISA cAMP assay kits, this homogenous cAMP assay kit does not require a wash step or the acetylation step. The assay is based on the competition for a fixed number of anti-cAMP antibody binding sites between the fluorescent cAMP tracer and non-labeled free cAMP. Free cAMP displaces the fluorescent cAMP tracer from the HRP-cAMP/anti-cAMP antibody complex. The anti-cAMP antibody is labeled with our trFluor™ Eu while the cAMP tracer contains our cAMP-trFluor™ 650. In the absence of cAMP, cAMP-trFluor™ 650 conjugate is bound to trFluor™ Eu-labeled anti-cAMP antibody exclusively to have a strong FRET. However, the unlabeled free cAMP in the test sample competes for the trFluor™ Eu-labeled anti-cAMP antibody conjugate, therefore inhibiting the binding of cAMP-trFluor™ 650 to the anti-cAMP antibody. The cAMP-trFluor™ 650 labeled cAMP tracer only has a fluorescence lifetime in nanoseconds while trFluor™ Eu-labeled anti-cAMP antibody-bound fluorescent cAMP tracer has a much longer fluorescence lifetime value due to the TR-FRET. The magnitude of FRET is proportional to the concentration of cAMP in a sample.

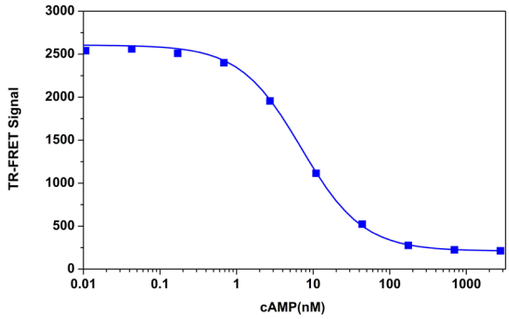

Figure 14. cAMP dose responses were measured with Screen Quest™ FRET No Wash cAMP Assay Kit (Cat# 36379) in a 96-well solid black plate. As low as 1 nM cAMP was detected in a 100 µL reaction.

trFluor™ Eu TRF Labeling Dyes

AAT Bioquest's trFluor™ Eu probes enable TRF for the assays that require high sensitivity. The trFluor™ Eu dyes have large Stokes shifts and extremely long emission half-lives when compared with more traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared with other time-resolved fluorescent probes, our trFluor™ Eu probes have relatively high stability, high emission yield and the ability to be linked to biomolecules with higher conjugation yields. Moreover, our trFluor™ Eu probes are insensitive to fluorescence quenching when conjugated to biological polymers such as

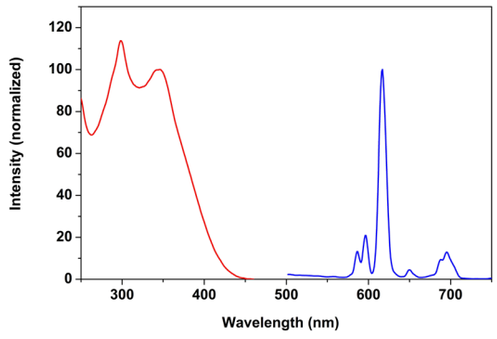

Figure 15. The excitation and emission spectra of trFluor™ Eu Goat Anti-Rabbit IgG Conjugate (Cat# 16668) in PBS buffer (pH 7.2).

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antibodies. To maximize the TR-FRET potential, trFluor™ Eu dyes are optimized to pair with APC, iFluor™ 647, TF5, Cy5®, DyLight™ 650 and Alexa Fluor® 647.

trFluor™ Tb TRF Labeling Dyes

AAT Bioquest's trFluor™ Tb dyes have large Stokes shifts and extremely long emission half-lives when compared with more traditional fluorophores such as Alexa Fluor® or cyanine dyes. Compared with other time-resolved fluorescent probes, our trFluor™ Tb probes have relatively high stability, high emission yield and ability to be linked to biomolecules with higher conjugation yield. Moreover, our trFluor™ Tb probes are insensitive to fluorescence quenching when conjugated to biological polymers such as antibodies. To maximize the TR-FRET potential, trFluor™ Tb dyes are optimized to pair with FITC, iFluor™ 488, TF2, DyLight™ 488 and Alexa Fluor® 488.

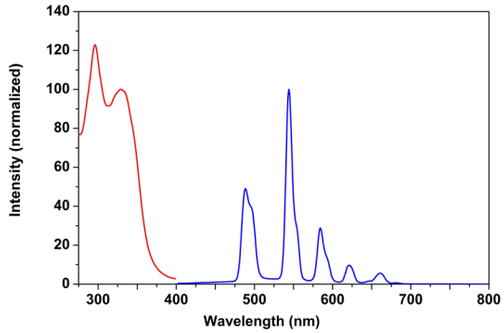


Figure 16. The excitation and emission spectra of trFluor™ Tb Goat Anti-Rabbit IgG Conjugate (Cat# 16669) in PBS buffer (pH 7.2).

Table 4. TR-FRET Assays and Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
1300	ReadiLink™ Rapid trFluor™ Eu Antibody Labeling Kit	2 labelings	346	617
1305	ReadiLink™ Rapid trFluor™ Tb Antibody Labeling Kit	2 labelings	330	544
36379	Screen Quest™ FRET No Wash cAMP Assay Kit	1 plate	390	650
36380	Screen Quest™ FRET No Wash cAMP Assay Kit	10 plates	390	650
16518	trFluor™ Eu Goat Anti-Mouse IgG (H+L)	100 µg	346	617
16755	trFluor™ Eu Goat Anti-Mouse IgG (H+L)	1 mg	346	617
16598	trFluor™ Eu Goat Anti-Mouse IgG (H+L) *Cross Adsorbed*	100 µg	346	617
16791	trFluor™ Eu Goat Anti-Mouse IgG (H+L) *Cross Adsorbed*	1 mg	346	617
16668	trFluor™ Eu Goat Anti-Rabbit IgG (H+L)	100 µg	346	617
16820	trFluor™ Eu Goat Anti-Rabbit IgG (H+L)	1 mg	346	617
16725	trFluor™ Eu Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	100 µg	346	617
16847	trFluor™ Eu Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	1 mg	346	617
1434	trFluor™ Eu Maleimide	100 µg	346	617
16925	trFluor™ Eu-Streptavidin Conjugate	100 µg	346	617
1433	trFluor™ Eu Succinimidyl Ester	1 mg	346	617
16519	trFluor™ Tb Goat Anti-Mouse IgG (H+L)	100 µg	330	544
16756	trFluor™ Tb Goat Anti-Mouse IgG (H+L)	1 mg	330	544
16599	trFluor™ Tb Goat Anti-Mouse IgG (H+L) *Cross Adsorbed*	100 µg	330	544
16792	trFluor™ Tb Goat Anti-Mouse IgG (H+L) *Cross Adsorbed*	1 mg	330	544
16669	trFluor™ Tb Goat Anti-Rabbit IgG (H+L)	100 µg	330	544
16821	trFluor™ Tb Goat Anti-Rabbit IgG (H+L)	1 mg	330	544
16726	trFluor™ Tb Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	100 µg	330	544
16848	trFluor™ Tb Goat Anti-Rabbit IgG (H+L) *Cross Adsorbed*	1 mg	330	544
1444	trFluor™ Tb Maleimide	100 µg	330	544
16926	trFluor™ Tb-Streptavidin Conjugate	100 µg	330	544
1443	trFluor™ Tb Succinimidyl Ester	1 mg	330	544

RNA Detection

Detecting and quantitating small amounts of RNA is extremely important for a wide variety of molecular biology procedures such as measuring yields of *in vitro* transcribed RNA and measuring RNA concentrations before performing Northern blot analysis, S1 nuclease assays, RNase protection assays, cDNA library preparation, reverse transcription PCR, and differential display PCR. The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm. The major disadvantage of the absorbance-based method is the interferences caused by proteins and other UV absorbing compounds.

The major challenge to analyze RNA in live cells is the interferences caused by DNA. To address the difficulties, AAT Bioquest has developed the StrandBrite™ RNA Green, an excellent RNA-selective probe that generates significantly enhanced green fluorescence upon binding to RNA. StrandBrite™ RNA Green readily gets into live cells. It has the excitation/emission of 490/540 nm. In the DNase digest test, no significant change of fluorescence intensity in fixed cells stained with StrandBrite™ RNA Green was observed. In contrast, after RNase digestion, the initial fluorescence signal decreased immediately. These results indicate that initial fluorescence signal was generated from the specific interaction of StrandBrite™ RNA Green with RNA in cells. Short exposure of live cells to antinomycin D did cause inhibition of RNA synthesis during 6 hours after drug removal in a dose-dependent manner. These data demonstrate that StrandBrite™ RNA Green is a sensitive RNA-selective dye for staining nucleolar RNA in live and fixed cells. StrandBrite™ RNA Green has less DNA interferences than the commonly used SYTO® RNASelect™ dye. Due to its excellent cell permeability and spectral properties, it has been successfully used for flow cytometric RNA analysis and fluorescence microscope in live cells.

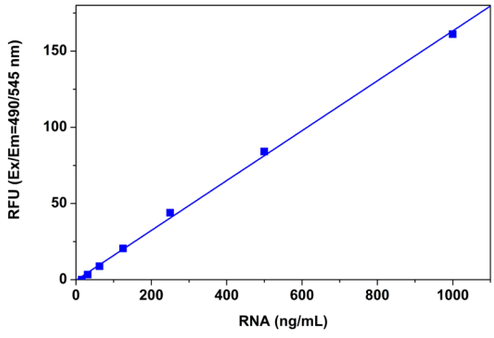
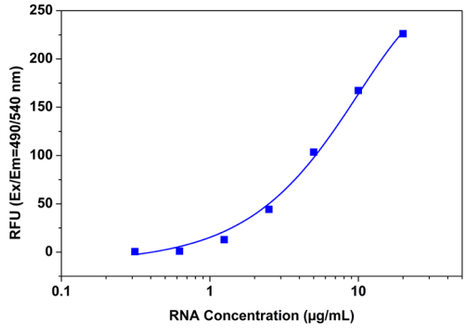


Figure 17. RNA dose responses were measured with StrandBrite™ Green Fluorimetric RNA Quantitation Kit (Cat# 17655) in a solid black 96-well microplate.

StrandBrite™ Green RNA Quantifying Reagent (Cat# 17610) is an ultrasensitive fluorescent nucleic acid stain for quantitating RNA in solution. StrandBrite™ Green RNA Quantifying Reagent can detect as low as 5 ng/mL RNA with a fluorescence microplate reader or fluorometer. StrandBrite™ Green Fluorimetric RNA Quantitation Kits (Cat# 17655, 17656 & 17657) include our StrandBrite™ RNA Green nucleic acid stain with optimized and robust protocols. They provide convenient methods for quantifying RNA in solutions.



Cell Navigator™ Live Cell RNA Imaging Kit (Cat# 22630) includes StrandBrite™ RNA Green as it specifically binds RNA in cells. Compared with commercial SYTO® RNASelect™ dye for RNA staining *in vivo*, StrandBrite™ RNA Green shows a brighter signal and much better selectivity to RNA. In addition, this kit can stain RNA in both living cells and fixed cells.

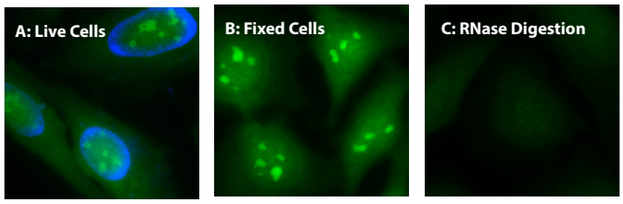


Figure 19. Fluorescence images of RNA staining in HeLa cells. **A:** Live cells were stained using Cell Navigator™ Live Cell RNA Imaging Kit (Green, Cat# 22630) and counter-stained with Hoechst 33342 (Blue, Cat# 17530). **B:** Cells fixed in methanol were stained using the same kit. **C:** After staining, fixed HeLa cells were incubated with 0.5 mg/mL RNase at 37 °C for 1 hour. Image of RNase digest test indicated the high selectivity of StrandBrite™ RNA Green. The green fluorescence signal was measured using a fluorescence microscope with FITC filter.

Table 5. RNA Detection Assays and Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22630	Cell Navigator™ Live Cell RNA Imaging Kit	100 tests	503	511
17656	StrandBrite™ Green Fluorimetric RNA Quantitation Kit	100 tests	500	525
17655	StrandBrite™ Green Fluorimetric RNA Quantitation Kit *Optimized for Microplate Readers*	1000 tests	500	525
17657	StrandBrite™ Green Fluorimetric RNA Quantitation Kit *High Selectivity*	100 tests	500	525
17610	StrandBrite™ Green RNA Quantifying Reagent	1 mL	500	525
17611	StrandBrite™ Green RNA Quantifying Reagent	10 mL	500	525

Fluorescent Calcium Indicators

Calcium acts as a universal second messenger in a variety of cells. Numerous functions of all types of cells are regulated by Ca^{2+}, thus calcium measurement is critical for various biological investigations. Since the 1920s, scientists have attempted to measure Ca^{2+}, but few were successful due to the limited availability of Ca^{2+} probes. The first reliable measurement of Ca^{2+} was performed by Ridgway and Ashley by injecting the photoprotein aequorin into the giant muscle fiber of the barnacle. Subsequently, in the 1980s, Tsien and colleagues produced a variety of fluorescent indicators. Among these indicators, Indo-1, Fura-2, Fluo-3 and Rhod-2 have been the most valuable dyes for measuring Ca^{2+} with a fluorescence instrument. In recent years, AAT Bioquest has introduced the most robust calcium probes: Fluo-8® and Cal-520®, both of which enable the high throughput screening of GPCR and calcium channel drug discovery targets through the convenient calcium detection. FLIPR® and FlexStation® instruments of Molecular Devices, FDSS®/µCELL of Hamamatsu and NOVOstar of BMG Technologies have further accelerated the high throughput measurement of calcium for GPCR and ion channel research.

Fluorescent probes that show spectral responses upon binding Ca^{2+} have enabled researchers to investigate changes in intracellular free Ca^{2+} concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Most of these fluorescent indicators are derivatives of BAPTA chelators that incorporate a PET system responsive to calcium. There are quite a few factors that need be considered when selecting a fluorescent Ca^{2+} indicator.

Among the visible light-excitable calcium indicators, Fluo-8®, Fluo-4, Fluo-3, Rhod-2 and Rhod-4™ are most commonly used. Fluo-8® indicators are widely used in flow cytometry and confocal laser-scanning microscopy. More recently, Fluo-8® AM has been extensively used for high throughput screening of GPCR targets. Fluo-8® is essentially nonfluorescent unless bound to Ca^{2+} and exhibits a quantum yield of ~0.15 in the presence of saturating Ca^{2+} and a K_d of 390 nM for Ca^{2+}. Cal-520® is by far the best 488 nm-excitable green fluorescent calcium indicator with a significantly improved signal/background ratio and intracellular retention.

Table 6. Classic Single Wavelength Fluorescent Calcium Indicators

Cat #	Product Name	Size	Ex (nm)	Em (nm)	K_d
21130	Cal-520®, AM	10x50 µg	492	514	320 nM
20511	Cal-590™, AM	10x50 µg	573	588	561 nM
21011	Fluo-3, AM *UltraPure grade*	1 mg	506	526	390 nM
21018	Fluo-3, Pentaammonium Salt	1 mg	506	526	390 nM
21017	Fluo-3, Pentapotassium Salt	1 mg	506	526	390 nM
21016	Fluo-3, Pentasodium Salt	1 mg	506	526	390 nM
20550	Fluo-4, AM	1 mg	494	516	345 nM
21080	Fluo-8®, AM	1 mg	494	517	389 nM
21064	Rhod-2, AM *UltraPure grade*	20x50 µg	549	578	570 nM
21067	Rhod-2, Tripotassium Salt	1 mg	549	578	570 nM
21068	Rhod-2, Trisodium Salt	1 mg	549	578	570 nM
21070	Rhod-5N, AM	1 mg	551	577	0.3 mM
21072	Rhod-5N, Tripotassium Salt	1 mg	551	577	0.3 mM

The long-wavelength Rhod-4™, Cal-590™ and Cal-630™ are valuable alternative Ca^{2+} indicators to the green fluorescent Fluo-8®, Fluo-4 and Fluo-3 for experiments in cells and tissues that have high levels of autofluorescence. Rhod-5N has a lower binding affinity for Ca^{2+} than any other BAPTA-based indicator (K_d = ~320 µM) and is suitable for Ca^{2+} measurements from 10 µM to 1 mM. Like the parent Rhod-2 indicator, Rhod-5N is essentially nonfluorescent in the absence of divalent cations and exhibits strong fluorescence enhancement with no spectral shift upon binding Ca^{2+}. Both Fluo and Rhod indicators are available as cell-impermeant potassium salts or as cell-permeant AM esters.

Dextran Conjugates of Calcium Indicators

Calcium measurement is critical for numerous biological investigations. Fluorescent probes that show spectral responses upon binding to calcium have enabled researchers to investigate changes in intracellular free calcium concentrations by using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Cells may be physically loaded with the cell-impermeant salt forms of these dextran-conjugated calcium indicators using patch pipettes or microinjection. The fluorescence signal from these cells is measured using fluorescence microscopy. The dextran forms of our calcium indicators show a dramatic reduction in both leakage and compartmentalization compared to the AM ester forms. Among the fluorescent calcium indicator dextran conjugates, Cal-520® dextran conjugates are the best choice due to their high fluorescence quantum yields and large fluorescence enhancement by calcium. Compared with Oregon Green® BAPTA-1 dextran, Cal-520® dextran exhibits much lower background and

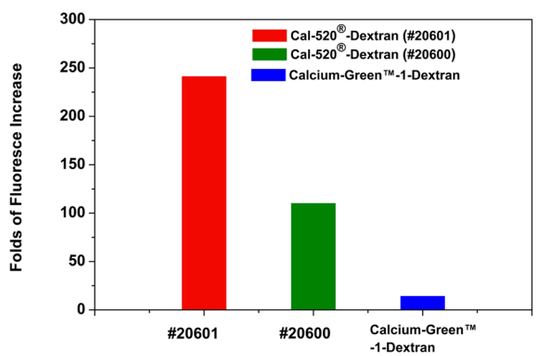


Figure 20. Fluorescence intensity increase of Cal-520® -Dextran (Cat# 20600), Cal-520® -Dextran (Cat# 20601) and Calcium-Green™ -1-Dextran upon binding to saturated amount of Ca^{2+}.

much larger calcium-induced fluorescence enhancement. Among the fluorescent calcium indicator dextran conjugates, Cal-590™ & Cal-630™ dextran conjugates might be better choices than other red fluorescent dextran conjugates due to their higher fluorescence quantum yields and larger fluorescence enhancement by calcium.

Table 7. Dextran Conjugates of Calcium Indicators

Cat #	Product Name	Size	Ex (nm)	Em (nm)
20600	Cal-520®-Dextran Conjugate *MW 3,000*	1 mg	492	514
20601	Cal-520®-Dextran Conjugate *MW 10,000*	5 mg	492	514
20508	Cal-590®-Dextran Conjugate *MW 3,000*	1 mg	573	588
20509	Cal-590®-Dextran Conjugate *MW 10,000*	1 mg	573	588
20545	Cal-630®-Dextran Conjugate *MW 3,000*	1 mg	608	626
20546	Cal-630®-Dextran Conjugate *MW 10,000*	1 mg	608	626

Cal Red™ R525/650

Cal Red™ R525/650 has been developed as a new 488 nm-excitable ratiometric fluorescence calcium indicator. Cal Red™ R525/650 is weakly fluorescent. Once it enters cells, the lipophilic AM blocking groups are cleaved by intracellular esterase, resulting in a negatively charged fluorescent dye retained well in cells with excitation close to 488 nm and two emissions at 525 nm and 650 nm. When cells are stimulated with a bioactive compound, the receptor initiates the release of intracellular calcium, which is chelated by Cal Red™ R525/650. The emission signal is increased at 525 nm and decreased at 650 nm when excited at 488 nm. The excitation and emission wavelengths of Cal Red™ R525/650 are compatible with common filter sets and cause minimal damage to cells, making it a robust tool for evaluating and screening GPCR agonists and antagonists, as well as calcium channel targets.

Key Features of Cal Red™ R525/650 :

- The excitation and emission wavelengths are compatible with common filter sets.
- Compared with Fura Red, it has significantly higher S/N ratio.
- Compared with Fura-2, it has minimal damage to cells and is more photostable.

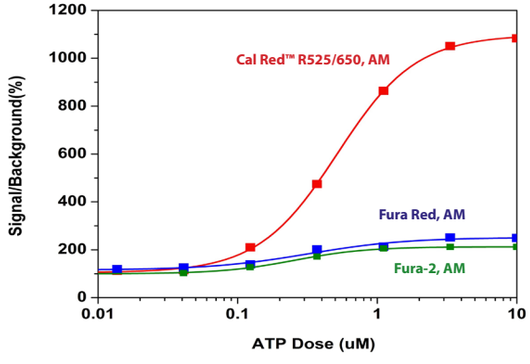


Figure 21. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with different Ca2+ indicators under the same conditions. ATP (50 µL/well) was added by FlexStation® 3 (Molecular Devices) to achieve the final indicated concentrations. (Red: Cal Red™ R525/650, AM, Cat# 20591; Blue: Fura Red, AM, Cat# 21046; Green: Fura-2, AM, Cat# 21023).

Table 8. Cal Red™ R525/620

Cat #	Product Name	Size	Ex (nm)	Em (nm)
20590	Cal Red™ R525/650, AM	1 mg	492	525/650
20591	Cal Red™ R525/650, AM	10x50 µg	492	525/650
20588	Cal Red™ R525/650, Potassium Salt	5x50 µg	492	525/650

Fluo-4 Calcium Indicators

Fluo-3 and Fluo-4 are most commonly used among the visible light-excitable calcium indicators. Fluo-4 is an analog of Fluo-3 with the two chlorine substituents replaced by fluorines, which results in increased fluorescence excitation at 488 nm and consequently higher fluorescence signal levels. Cells may be loaded with the AM ester forms of these calcium indicators by adding the dissolved indicator directly to dishes containing cultured cells. AAT Bioquest offers Fluo-4 AM in the best quality with the best price.

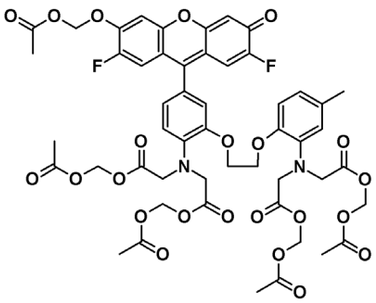


Figure 22. The chemical structure of Fluo-4 AM (Cat# 20552).

Fluo-3 AM and Fluo-4 AM are only moderately fluorescent in live cells upon esterase hydrolysis, and require harsh cell loading conditions to maximize their cellular calcium responses. Fluo-8® and Cal-520® calcium dyes have been developed to improve cell loading and calcium response while maintaining the convenient Fluo-3 and Fluo-4 spectral wavelengths of maximum excitation @ ~490 nm and maximum emission @ ~520 nm.

Table 9. Fluo-4 Calcium Indicators

Cat #	Product Name	Size	Ex (nm)	Em (nm)
20552	Fluo-4, AM *Ultrapure Grade*	5x50 µg	494	516
20551	Fluo-4, AM *Ultrapure Grade*	10x50 µg	494	516
20550	Fluo-4, AM *Ultrapure Grade*	1 mg	494	516
20556	Fluo-4, Pentapotassium Salt	10x50 µg	494	516
20555	Fluo-4, Pentapotassium Salt	1 mg	494	516

Calcein-Based Cell Viability Assays

Cell viability assays are an essential part of biological research. For example, cell viability assays are used in cancer studies to determine the cytotoxicity of a particular drug on a subset of cells, and by extension, provide a measure of the proliferation of cancerous cells. Cell viability assays are also critical in the study of cytomembranes, whether it be a mitochondrial membrane, a vesicular membrane, or the membrane of the cell itself. Many researchers are generally interested in quantifying the number of viable cells after an experimental procedure, to gauge the effect (or lack thereof) of such procedures on cell viability. Given the importance of such assays, therefore, many different methods have been developed to quantify cell viability. One particularly prevalent compound is calcein.

Calcein is a fluorescein derivative which was originally implemented as a calcium indicator. It has since been developed for use in cell viability assays. To deliver the fluorescent calcein probes into cells, a hydrophobic group, such as acetomethoxy (AM), is attached. With this reaction, calcein becomes calcein AM- a hydrophobic, non-fluorescent compound. This form of calcein allows it to readily pass through lipid membranes, whether it'd be a vesicular membrane or the cell membrane itself. Then to reach its active state, calcein AM is hydrolyzed by esterases which remove the acetomethoxy, returning the probe back to its calcein form. In this new state, the probe becomes hydrophilic, which allows it to be retained in the cell. Furthermore, it gains a strong fluorescence, which maximally excites at 495 nm and emits at 515 nm. Since calcein AM is a fluorometric assay, it has both a greater sensitivity and a greater range of sensitivity than colorimetric assays, such as MTT.

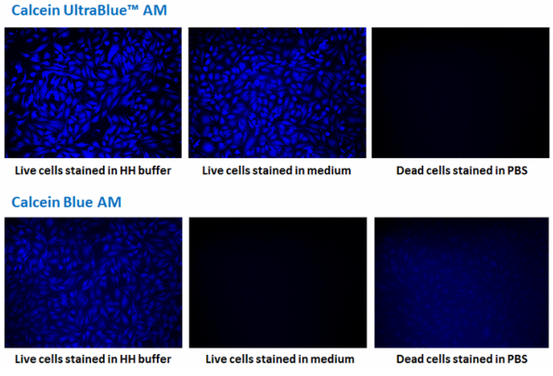


Figure 23. Fluorescence images of HeLa cells stained with Calcein UltraBlue™ AM (Upper row, Cat# 21908) or Calcein Blue AM (Lower row, Cat# 22007) in a Costar black wall/clear bottom 96-well plate. Left: Live HeLa cells in HH buffer; Middle: Live HeLa cells in medium; Right: Fixed HeLa cells.

Aside from its good solubility and greater sensitivity, calcein AM also benefits from having low cytotoxicity during its use. This derives from the fact that very little calcein is needed to achieve bright imaging results. Since less calcein AM is needed, the impact on cellular functions, such as proliferation, and organelle structures, such as mitochondria, is minimized. Additionally, the turnaround time is very short for calcein AM assays, meaning that cytotoxicity of calcein is further reduced. Unlike some assays, which have a procedure time of four to six hours, calcein based assays can be completed in less than two hours. Lastly, one important benefit calcein has is that it can be used in multicolor analysis. Dyes such as CytoCalcein™ Violet 450, CytoCalcein™ Violet 500, CytoCalcein™ Blue 550 and CytoCalcein™ Blue 600 have been developed for flow cytometric applications. CytoCalcein™ dyes exhibit similar biological properties to calcein AM. They are optimized for the excitation wavelengths of a variety of flow cytometers, providing additional colors for flow cytometric analysis of live cells. CytoCalcein™ Violet 450 and CytoCalcein™ Violet 500 are well excited by violet lasers (405 nm) and emit fluorescence at 450 nm and 500 nm respectively. CytoCalcein™ Blue 550 and CytoCalcein™ Blue 550 are well excited by blue lasers (488 nm) and emit fluorescence at 550 nm and 600 nm respectively. The wealth of colors allows for multiple variables to be probed at once. For example, calcein can be used in conjunction with GFP to probe a cell in two dimensions. Such multi-color analysis cannot be achieved with colorimetric assays such as MTT and is a unique property of calcein based dyes.

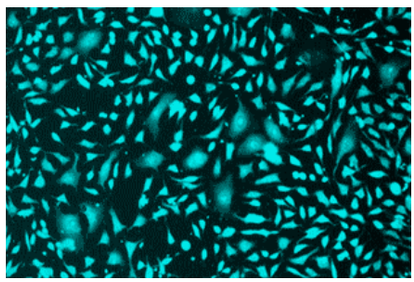


Figure 24. Image of CPA cells in a 96-well Costar black wall/clear bottom plate stained with CytoCalcein™ Violet 500 (Cat# 22013, Ex/Em = 405/500 nm, 405 Violet filter).

Table 10. Calcein-Based Cell Viability Probes

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22001	Calcein *UltraPure Grade*	10 mg	495	515
22002	Calcein, AM	1 mg	495	515
22004	Calcein, AM *UltraPure grade*	20x50 µg	495	515
22003	Calcein, AM *UltraPure grade*	1 mg	495	515
22006	Calcein Blue	25 mg	360	445
22007	Calcein Blue, AM	1 mg	360	445
21902	Calcein Deep Red™	1 mg	646	659
22010	Calcein Deep Red™ Acetate	1 mg	646	659
22009	Calcein Orange™ Diacetate	1 mg	525	550
22008	Calcein Orange™ Sodium Salt	1 mg	525	550
21900	Calcein Red™, AM	1 mg	560	574
21901	Calcein Red™ Sodium Salt	1 mg	560	574
21908	Calcein UltraBlue™, AM	10x50 µg	360	445
21909	Calcein UltraBlue™ Sodium Salt	1 mg	360	445
22012	CytoCalcein™ Violet 450 *Excited at 405 nm*	1 mg	408	450
22013	CytoCalcein™ Violet 500 *Excited at 405 nm*	1 mg	410	500

Fluorescence Live Cell Imaging

The endoplasmic reticulum (ER) is a type of organelle in the cells of eukaryotic organisms that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae. The membranes of the ER are continuous with the outer nuclear membrane. ER occurs in most types of eukaryotic cells, but is absent from red blood cells and spermatozoa.

Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit 22635 uses our ER Tracer™ Green as an ER marker. ER Tracer™ Green stain is a cell-permeant fluorescent dye that is highly selective for ER. This stain consists of a green fluorescent dye and ER binder that selectively bind to ER in most of cell types. For some cells, ER Tracer™ Green may not selectively bind to ER. ER Tracer™ Green has spectral properties essentially identical to FITC, making this kit convenient with the FITC filter set.

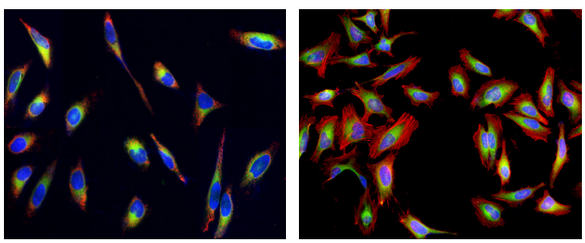


Figure 25. Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black wall/clear bottom plate. Left: Live cells were stained with Cell Navigator™ Live Cell Endoplasmic Reticulum Staining Kit (Cat# 22635, Green), mitochondria dye MitoLite™ Red FX600 (Cat# 22677, Red) and nuclei stain Hoechst 33342 (Cat# 17530, Blue). Right: Live cells stained with Kit 22635 (Green) were fixed with 4% formaldehyde, and labeled with F-actin dye Phalloidin-iFluor™ 594 (Cat# 23122, Red) and nuclei stain DAPI (Cat# 17507, Blue).

Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit 22636 uses our ER Tracer™ Red as an ER marker. ER Tracer™ Red stain is a cell-permeant fluorescent dye that is highly selective for ER. This stain consists of a red fluorescent dye and ER binder that selectively bind to ER in most of cell types. For some cells, ER Tracer™ Red may not selectively bind to ER. ER Tracer™ Red has spectral properties essentially identical to Texas Red®, making this kit convenient with the Texas Red® filter set.

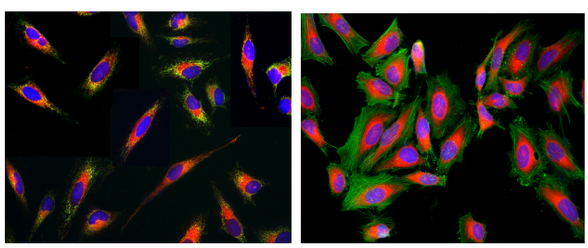


Figure 26. Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black wall/clear bottom plate. Left: Live cells were stained with Cell Navigator™ Live Cell Endoplasmic Reticulum Staining Kit (Cat# 22636, Red), mitochondria dye MitoLite™ Green (Cat# 22675, Green) and nuclei stain Hoechst 33342 (Cat# 17530, Blue). Right: Live cells stained with Kit 22636 (Red) were fixed with 4% formaldehyde, then labeled with F-actin dye Phalloidin-iFluor™ 488 (Cat# 22661, Green) and nuclei stain DAPI (Cat# 17507, Blue).

Table 11. Endoplasmic Reticulum (ER) Staining Assays

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22635	Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit *Green Fluorescence*	100 tests	503	511
22636	Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit *Red Fluorescence*	100 tests	589	620

The cell membrane (plasma membrane) is a thin semi-permeable membrane that separates the interior of all cells from the environment. Cell membranes are involved in a variety of cellular processes, such as cell adhesion, ion conductivity and cell signaling, and serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracelluar cytoskeleton.

Cell Navigator™ Cell Plasma Membrane Staining Kits (Cat# 22680 & 22681) provide a fast and uniform labeling of the plasma membrane without the cell-type differences exhibited by lectins. The kits may be used as a segmentation tool for HCS (high-content screening). They can also be used to stain cellular plasma membranes for standard fluorescence microscopy. The cell membrane probes used in the kits survive fixation, but not permeabilization, so they are not suitable for experiments that also involve probing internal targets via antibodies.

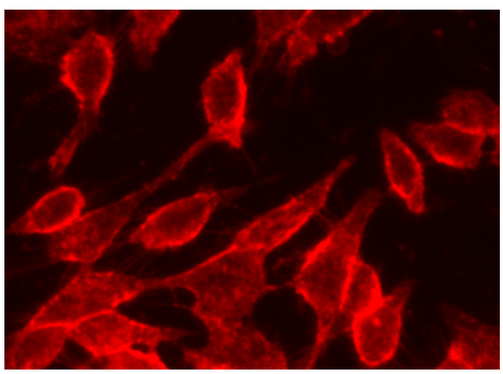


Figure 27. The fluorescence image of HeLa cells stained with Cell Navigator™ Cell Plasma Membrane Staining Kit (Cat# 22680) in a 96-well black wall/clear bottom plate. The cells were imaged using a fluorescence microscope with a TRITC filter.

Table 12. Cell Plasma Membrane Staining Assays

Cat #	Product Name	Size	Ex (nm)	Em (nm)
22680	Cell Navigator™ Cell Plasma Membrane Staining Kit *Orange Fluorescence*	500 tests	555	575
22681	Cell Navigator™ Cell Plasma Membrane Staining Kit *Red Fluorescence*	500 tests	650	670

International Distributors

Austria:
Biomol GmbH
Email: info@biomol.de
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Email: info@assaymatrix.com
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