MOLECULAR DEVICES

POWER *of* SpectraMax M

The industry standard
for microplate readers!

Introduction

For nearly 20 years, scientists have relied on the industry standard, the SpectraMax® M Series Multi-Mode Microplate Readers, for their consistent performance, reliability and durability.

Reader modes are customized to your specific applications with options to upgrade easily. All configurations offer a triple-mode cuvette port, accurate temperature control, microplate shaking and comprehensive data management using our SoftMax® Pro Microplate Data Acquisition and Analysis Software. Enhanced validation and verification procedures are automated by partnering validation plates and software to QC the readers quickly.

Contents

Power of SpectraMax M 1

Performance

Scientists have published over 20,000 peer-reviewed publications using the SpectraMax M Series Multi-Mode Microplate Readers. Each M Series reader reads UV and visible absorbance and fluorescence intensity measurements on microplates and cuvettes. Choose the reader that gives you the most empowering combination of features for your lab: UV and visible absorbance, fluorescence, glow luminescence, fluorescence polarization, TRF and HTRF.

All M Series readers accept cuvettes and 6-, 12-, 24-, 48-, 96-, and 384-well microplates. Other standard features include spectral scanning across the wavelength range in 1 nm increments, up to six absorbance wavelengths per read, and up to four wavelength pairs per read for other read modes.

- Patented AutoPMT Optimization System
- Patented PathCheck Pathlength Measurement Technology
- Comprehensive IQ/OQ reader validation services for GLP/GMP labs
- Validation plates for absorbance, fluorescence, and luminescence modes
- Easy automation interface
- Optimized assay kits

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Reliability

In May 2011, Molecular Devices SpectraMax M5e reader, chosen for its durability, became the first microplate reader in space. NanoRacks added it to their line of commercial research hardware on the International Space Station, providing researchers the ability to conduct microplate reader experiments in microgravity for the first time. They reconfigured the SpectraMax M5e, one of Molecular Devices most reliable and feature-rich microplate readers, to operate comfortably in the zero-gravity environment of the space station. Upgrades to the original platform include temperature control and the ability to configure every aspect of the researcher's experiment from an Earth-based workstation using SoftMax Pro, the industry's leading data acquisition and analysis tool.

Photos are courtesy of NanoRacks, LLC and NASA.

NanoRacks re-launched the reconfigured SpectraMax M5e reader back to the International Space Station on the SpaceX Commercial Resupply Mission-9 on July 18, 2016.

View Video | Read NanoRacks Press Release

Automated instrument validation

SpectraTest line of validation plates

The SpectraTest® ABS2, FL1, and LM1 Validation Packages from Molecular Devices provide automated, comprehensive, and traceable validation of optical performance, plus automatic verification of our microplate readers.

- NIST traceability
- Recertification by our ISO 17025 accredited laboratory
- Absorbance validation (ABS1)
- Fluorescence validation (FL1)
- Luminescence validation (LM1)
- Automated testing
- Preconfigured validation protocols available in SoftMax Pro Software

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IQ/OQ/PM Services

Qualifying your Molecular Devices microplate readers in GLP or GMP environments can now be done with greater reliability, security, and convenience. Presenting Compliance Assurance Digital IQ/OQ/PM Services, a unique qualification solution that preserves the documentation of legacy services in a digital and compliant format that can be accessed remotely.

Compliance Assurance Instrument Qualification

During each onsite Qualification, a trained Molecular Devices Service Engineer verifies and digitally documents instrument operation in accordance with IQ/OQ specifications. All qualification and preventive maintenance protocols are automated to expedite each service, while maintaining the integrity of your data and analysis results.

The service includes:

- Installation Qualification (IQ) verifies and documents that all necessary components required for operation are received and properly installed in accordance with Molecular Devices ISO 17025 certification requirements.
- Operational Qualification (OQ) tests the mechanical, electrical, and optical components of each instrument to verify proper operating functions in accordance with manufacturer specifications.
- Preventive Maintenance (PM) verifies each instrument meets operational specifications through comprehensive, multipoint inspection. Potential issues are proactively addressed, ensuring each instrument is maintained at optimal operational performance.

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GxP compliance

SoftMax Pro GxP Compliance Software extends our leading data acquisition and analysis solution into regulated laboratories working under GMP, GLP, 21 CFR Part 11, and other similar guidelines for secure electronic records.

Secure, traceable, electronic recordkeeping

- Controlled user access through a granular permission structure and unique logins
- Electronic signature support for verification, authorization, and approval
- Audit trails to document the history of user actions for each data file
- Local and remote administration of user accounts for straightforward deployment

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Save time and reduce cost

- Extensive suite of tools available for validation can reduce the cost and time of validation as compared to using multiple platforms to collect and analyze data by 50%
- Provides end-to-end chain of custody from capture through analysis to validation of data

Trusted

- SoftMax Pro 7 GxP is our 4th generation software with compliance tools
- Satisfied customers include all 50 of the top 50 global pharmaceutical companies

Software validation

For researchers working in GLP or GMP laboratories, the SoftMax Pro Software Validation Package provides the most comprehensive documentation and tools available to validate GxP administrator features, software operation and analysis functions for microplate reader instrumentation.

- Reduces validation time from 6 months to 3 days
- Validation tools for parallel line analysis, 4-P and 5-P curve fits
- Comprehensive tests for routine assay calculations
- Ready-to-use data for OQ confirmation tests
- Printable IQ/OQ documents for GLP/GMP paper trail

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Highlighted applications 2

Cell-based measurement of ERK1/2 phosphorylation with THUNDER™ TR-FRET assay

The MAPK/ERK cell signaling pathway begins with the activation of a receptor tyrosine kinase located at the cell surface, involves a series of kinase phosphorylation steps (kinase cascade), and culminates in the activation of transcription factors that effect changes in gene expression. ERK1 and ERK2, also referred to as ERK1/2, are phosphorylated as part of this pathway, and go on to phosphorylate and activate other targets, including transcription factors involved in cell proliferation and differentiation. Disruption or dysregulation of the pathway can lead to the development of a variety of cancers[1].

Benefits

- Highly robust homogeneous (no-wash) assay with simple and fast assay workflow
- Highly sensitive assay generating well-defined, complete concentration-response curves without the need to serum starve the cells
- EC_{50} and IC_{50} values in agreement with published data
- Suitable for both academic and drug discovery applications

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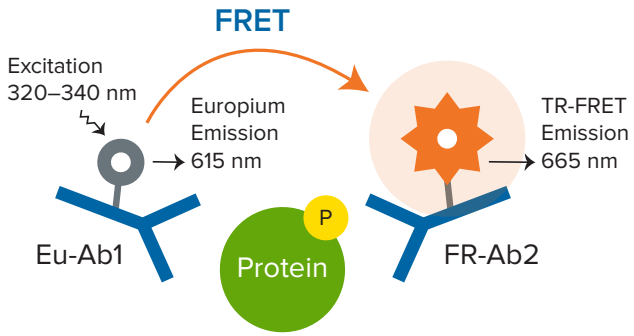
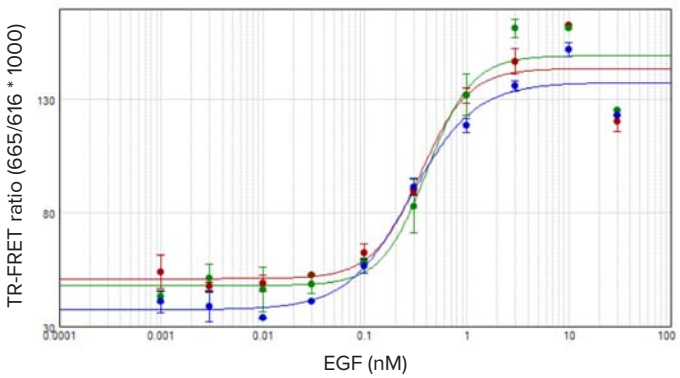
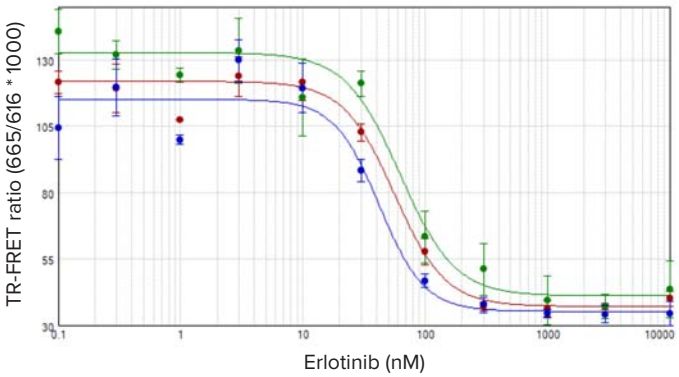


THUNDER TR-FRET sandwich immunoassay. Binding of both the Eu-Ab1 and FR-Ab2 to the analyte enables a transfer of energy from the Europium chelate to the acceptor fluorophore, resulting in signal at 665 nm that is detected using a microplate reader with time-resolved detection mode.



Concentration-dependent ERK1/2 (T202/Y204) phosphorylation induced by EGF. Results obtained with SpectraMax iD5 (red), i3x (green), and M5e (blue) readers were plotted using a 4-parameter logistic, and EC_{50} values of 0.28 nM to 0.41 nM were calculated using SoftMax Pro Software.



Inhibition of EGF-induced phosphorylation of ERK1/2 (T202/Y204) by Erlotinib. Data obtained with SpectraMax iD5 (red), i3x (green), and M5e (blue) readers were plotted using a 4-parameter logistic in SoftMax Pro Software, and IC_{50} values of 42 to 63 nM were calculated from the curve.

Assess virus neutralization with a rapid, HTS-friendly assay

The worldwide COVID-19 pandemic caused by the SARS-CoV-2 virus has necessitated the fast-tracked development of many research tools for understanding this virus's pathogenesis, as well as vaccine discovery and development. Assays for monitoring the immune response to infection by and vaccination against the virus are important to COVID-19 research. As neutralizing antibodies are key biomarkers of immune response and vaccine efficacy, levels of neutralizing antibodies in patient serum samples is an important parameter to be able to monitor efficiently.

Benefits

- Rapid, easy assay setup with an ELISA workflow
- No BSL-3 or cell culture required
- Results consistent with data from plaque reduction neutralization test

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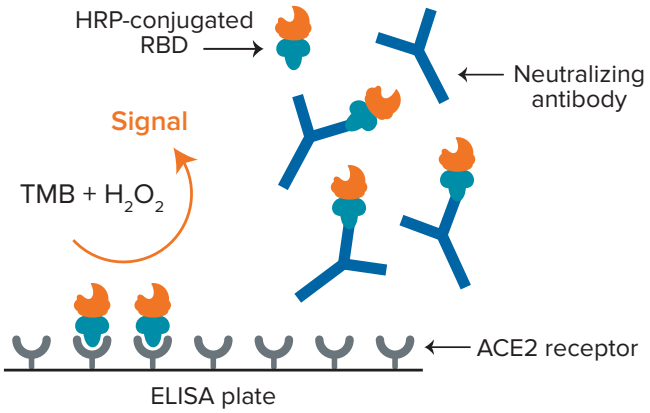
PRNT assay (BSL3 lab)	sVNT kit (BSL2 lab)
Cell and virus preparation	Incubate testing samples with HRP-RBD for 30 minutes
Incubate testing samples with virus for 1 hour	Add the above mixture to the wells pre-coated with ACE2 for 15 minutes
Add the above mixture to Vero-E6 cells and incubate for 1 hour	Wash wells
Overlay 1% agarose and incubate for 2 days	Add substrate and incubate for 15 minutes
Fix cells and stain	Add s top solution and read
Count plaques	
Total assay time = 2.5 days	**Total assay time = 1 hour**

Comparison of PRNT and sVNT kit workflows. The sVNT assay can be completed in one hour in a BSL-2 lab, while PRNT requires live SARS-CoV-2 virus and cumbersome cell culture techniques, taking more than two days at a BSL-3 lab.



SARS-CoV-2 sVNT method. The assay uses an ELISA format that enables detection of disruption of RBD binding to ACE2 by neutralizing antibodies in a test sample.

Sensitive fluorescent quantitation of DNA with the Quant-iT PicoGreen dsDNA Assay Kit

Double-stranded DNA is typically quantitated in microplate readers by measuring the absorbance of the DNA solution at 260 nm. However, this method is only able to measure down to about 250 ng/mL on a typical absorbance microplate reader. For biological applications involving small samples, such as next-generation sequencing and quantitation of DNA amplification products, more sensitive methods are needed. The Quant-iT PicoGreen dsDNA Assay Kit from Thermo Fisher Scientific is more specific for DNA and is about 1000 times more sensitive than traditional absorbance methods. The dynamic range of this assay in microplate format, as stated in the product manual, is from 250 pg/mL to 1000 ng/mL with a single dye concentration. Here, we demonstrate that with Molecular Devices SpectraMax® microplate readers and the Quant-iT PicoGreen assay, users can reliably measure concentrations as low as 50 pg/mL of double-stranded DNA.

Benefits

- Sensitive fluorescent quantitation of DNA down to 50 pg/mL
- Linear dynamic range spanning over four orders of magnitude
- Easy analysis of results with preconfigured protocol in SoftMax Pro Software

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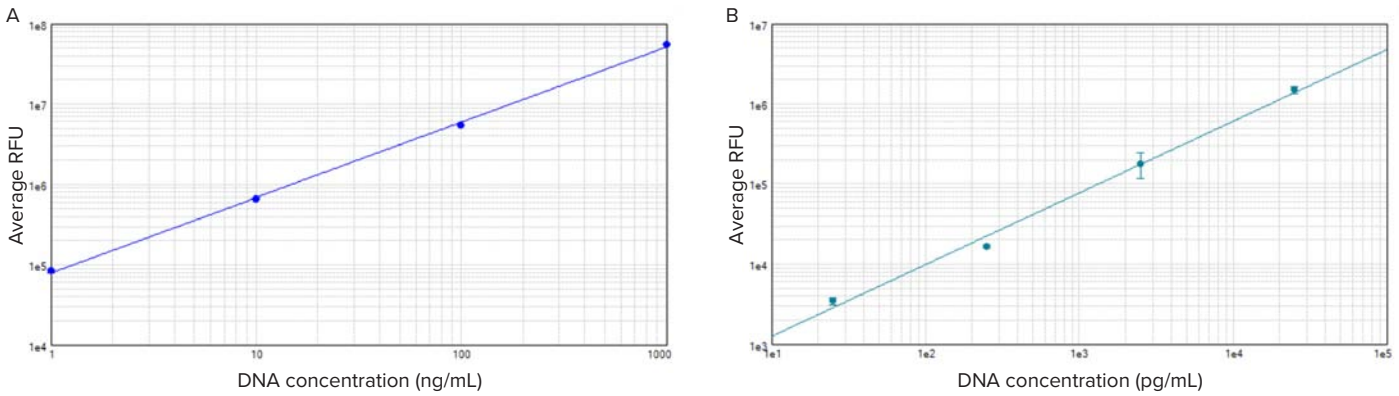

High-range (A) and low-range (B) standard curves. Curves were plotted using the log-log curve fit in SoftMax Pro Software (both curves, $r^2 = 0.99$).

MycoAlert Mycoplasma Detection Assays

Mycoplasma, the smallest and simplest of the prokaryotes, are common contaminants of cell cultures. It cannot be detected by simply examining cell cultures under a microscope, therefore, a sensitive and reliable assay is needed to determine whether contamination is present. Traditional detection methods involve time-consuming staining or PCR protocols, and the results can be difficult to interpret. The MycoAlert™ Assay and MycoAlert PLUS Assay from Lonza provide a rapid and convenient way to detect viable mycoplasma in cell cultures using a luminescence microplate reader. Here, we demonstrate how our microplate readers with the luminescence detection mode provide superior sensitivity and ease of use for reliable mycoplasma detection using MycoAlert assays.

Benefits

- Sensitive, reliable detection of mycoplasma contamination
- Simple add-and-read method for rapid results
- Easy interpretation of results

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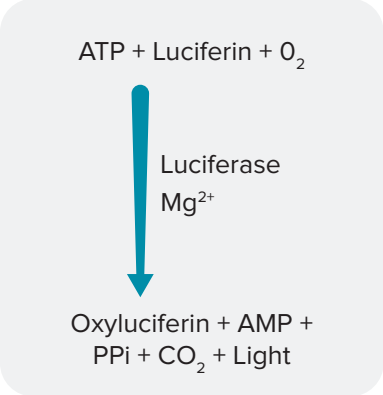
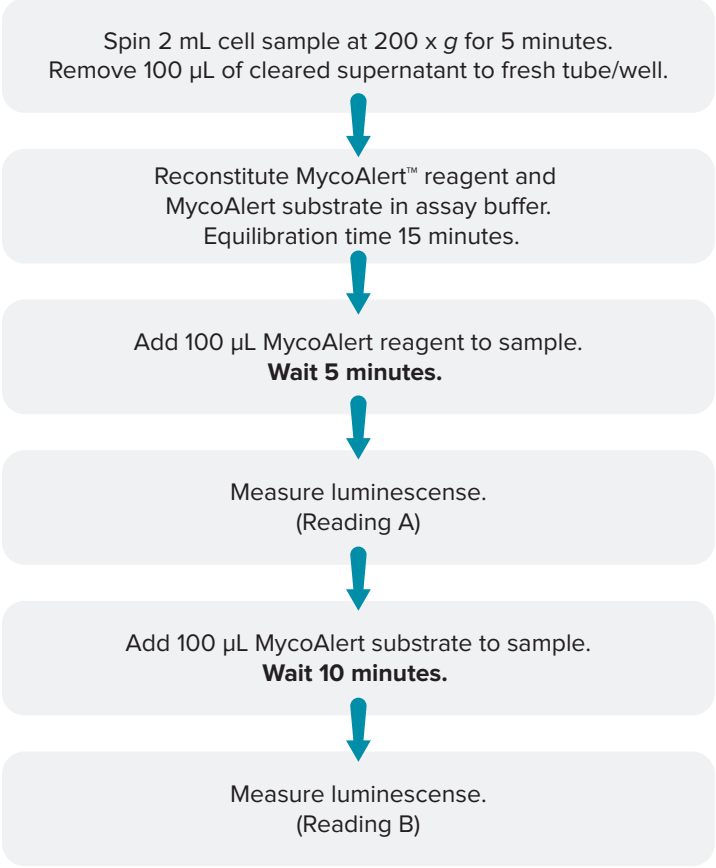


Bioluminescent reaction to detect ATP.



MycoAlert assay workflow. The luminescence measurement for Reading B is divided by Reading A to obtain a ratio indicative of the presence (ratio > 1.2) or absence (ratio < 0.9 with the MycoAlert Assay and < 1.0 with MycoAlert PLUS) of mycoplasma.

Noninvasive measurement of fluorescent proteins in live cells

Fluorescent proteins are popular tools for monitoring biological events in vivo. They are stable, have minimal toxicity and have the ability to generate visible fluorescence *in vivo* without the need for external cofactors. They can be monitored by fluorescent microplate readers which offer a preferable, more convenient and higher-throughput detection method. Here, we show how our fluorescent microplate readers can easily detect fluorescent proteins in intact adherent cells.

Benefits

- Easily and noninvasively measure fluorescent proteins in living cells
- Tune wavelengths to get optimal results for each individual fluorophore
- Read from the bottom for best sensitivity

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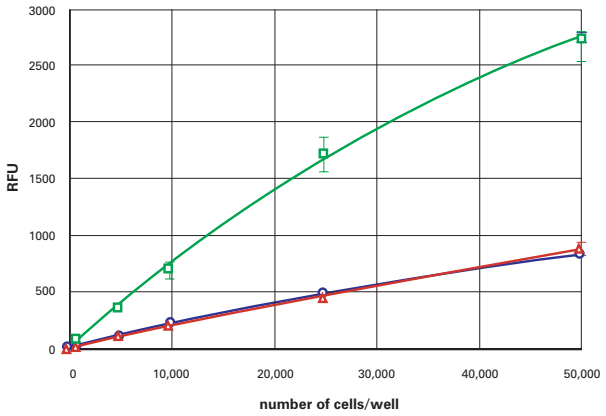
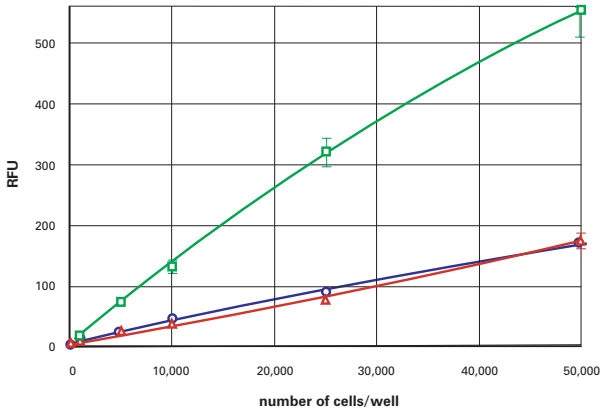
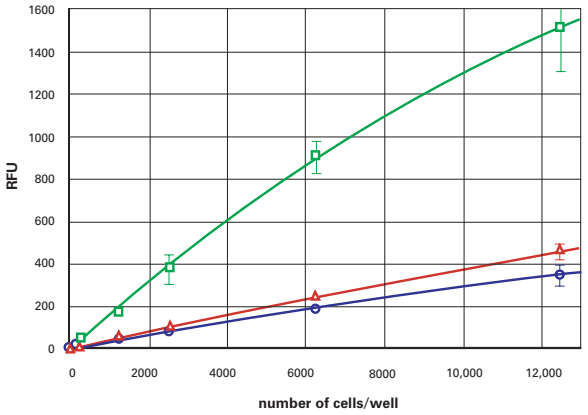


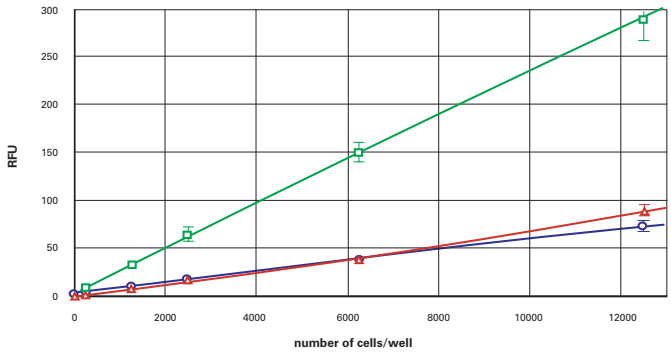
96-well fluorescent cell dilution series, bottom-read. Dilution series for ZsGreen (green), AcGFP (blue) and DsRed (red) transfected cells in 96-well plates, read from the bottom.



96-well fluorescent cell dilution series, top-read. Dilution series for ZsGreen (green), AcGFP (blue) and DsRed (red) transfected cells in 96-well plates, read from the top.



384-well fluorescent cell dilution series, bottom-read. Dilution series for ZsGreen (green), AcGFP (blue) and DsRed (red) transfected cells in 384-well plates, read from the bottom.



384-well fluorescent cell dilution series, top-read. Dilution series for ZsGreen (green), AcGFP (blue) and DsRed (red) transfected cells in 384-well plates, read from the top.

Measuring cell proliferation using the CyQUANT Cell Proliferation Assay

Quantitation of cell proliferation using fluorescence allows one to easily monitor the effects of drugs and other experimental treatments on cell growth. The CyQUANT Cell Proliferation Assay Kit from Life Technologies is a sensitive, rapid and convenient way to quantitate cell growth using a fluorescence microplate reader. CyQUANT GR dye binds to cellular nucleic acids, allowing cell numbers to be calculated from a standard curve. Because DNA-to-RNA ratios can vary over the course of the cell cycle, the CyQUANT kit allows users to determine cell numbers using RNase-digested cell lysates and a nucleic acid standard curve.

Benefits

- Rapid and convenient quantitation of cell growth
- Sensitive detection of as few as 50 cells
- Easy data analysis with preconfigured SoftMax Pro Software protocol

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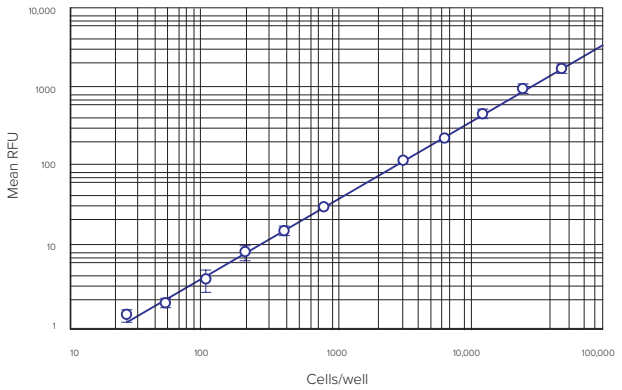
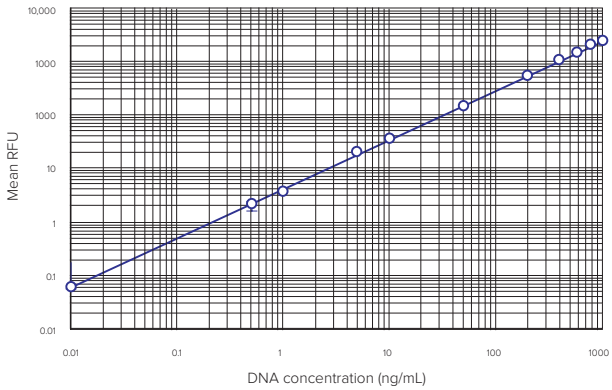
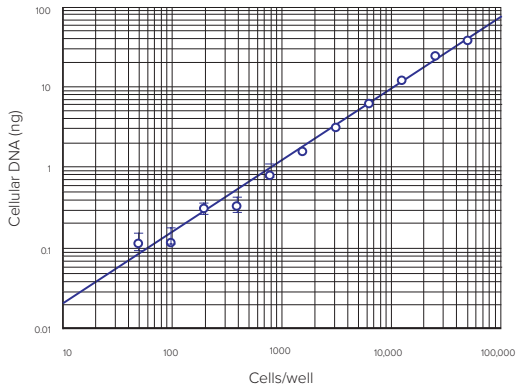
Here we describe how to use the CyQUANT kit with SpectraMax microplate readers and SoftMax Pro Software. Two methods are detailed. In the first, cellular proliferation is quantitated using a cell-based standard curve. In the second, cellular proliferation is quantitated using RNase-treated cell samples and a DNA standard curve.



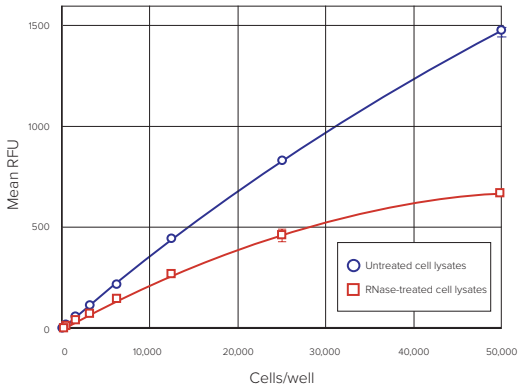
Cell-based standard curve. Cell densities (from 25 to 50,000 cells per well) were assayed.



DNA standard curve. DNA standard curve obtained using the CyQUANT assay kit.



Cellular DNA vs. cells-per-well. Cellular DNA concentration vs. number of RNase-treated cells per well.



Comparison between RNase-treated and untreated cells. RFU vs. Cells/well for RNasetreated cells compared to untreated cells. Blue circles, untreated cell lysates; red squares, RNasetreated cell lysates.

IMAP Phosphodiesterase and Kinase Assays

Based on the specific, high-affinity interaction of phospho groups with trivalent metal-containing nanoparticles (beads), IMAP is a generic, non-antibody-based platform to assess kinase, phosphatase, and phosphodiesterase activity. An enzyme reaction is performed using a fluorescently-labeled substrate. Addition of the IMAP® Binding System stops the enzyme reaction and initiates binding of the beads to phosphorylated substrates. Binding of the substrate to the beads, which correlates to enzyme activity, can be detected using either FP or TR-FRET as a readout. The kits are optimized for our microplate readers.

Benefits

- Complete assay system for screening kinases, phosphatases, and phosphodiesterases
- Robust fluorescence signal gives reliable results with good Z' factors
- Homogeneous and amenable to miniaturization for greater cost savings
- Available in both FP and TR-FRET detection modes to meet users' screening needs

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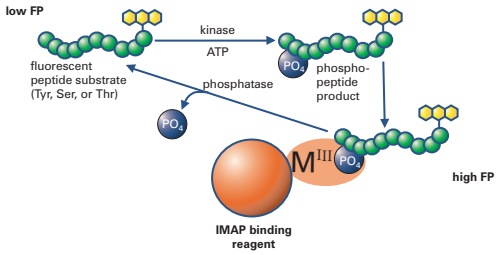
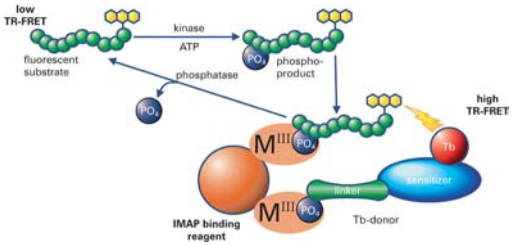
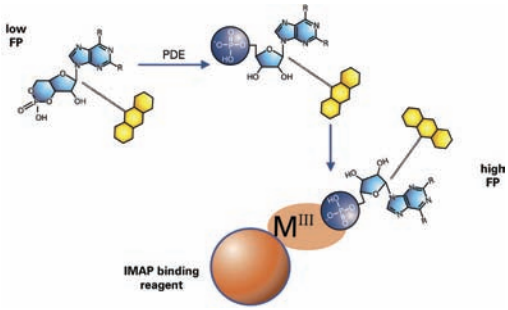


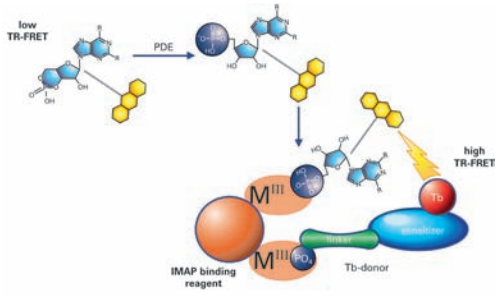
IMAP FP generic kinase and phosphatase assays. IMAP principle using FP readout: Binding Solution is added after the kinase reaction using a fluorescently labeled peptide. The small phosphorylated fluorescent substrate binds to the large M(III)-based nanoparticles which reduces the rotational speed of the substrate and thus increases its polarization.



IMAP TR-FRET generic kinase and phosphatase assays. IMAP principle using TR-FRET readout: Binding Solution is added after the kinase reaction using a fluorescently labeled peptide or protein. In this system, the nanoparticle is spiked with a Terbium (Tb)-Donor molecule. By binding to the spiked M(III)-based nanoparticles, the phosphorylated fluorescent substrate comes into close proximity with the Tb-Donor, which allows measurement of the TR-FRET between the Tb-Donor and the phosphorylated, fluorescent substrate.



IMAP FP generic phosphodiesterase assays. IMAP principle using FP readout: Binding Solution is added after the phosphodiesterase reaction using a fluorescently labeled substrate. The small phosphorylated fluorescent substrate binds to the large M(III)-based nanoparticles which reduces the rotational speed of the substrate and thus increases its polarization.



IMAP TR-FRET generic phosphodiesterase assays. IMAP principle using TR-FRET readout: Binding Solution is added after the phosphodiesterase reaction using a fluorescently labeled substrate. In this system, the nanoparticle is spiked with a Terbium (Tb)-Donor molecule. By binding to the spiked M(III)-based nanoparticles, the phosphorylated fluorescent substrate comes into close proximity with the Tb-Donor, which allows measurement of the TR-FRET between the Tb-Donor and the phosphorylated, fluorescent substrate.

HTRF cAMP HiRange Assay

HTRF® is a versatile technology developed by Cisbio Bioassays for detecting biomolecular interactions. It combines fluorescence resonance energy transfer (FRET) technology with time-resolved (TR) measurement of fluorescence, allowing elimination of short-lived background fluorescence. Here, we show how the SpectraMax microplate readers are used to perform robust, high-throughput HTRF assays with excellent Z' factors and highly reproducible EC_{50} values. Data acquisition and analysis are simplified using SoftMax Pro Software with preconfigured HTRF protocols.

Benefits

- Highly robust homogeneous assay
- Z' factor ≥ 0.9
- Streamlined and stable for HTS

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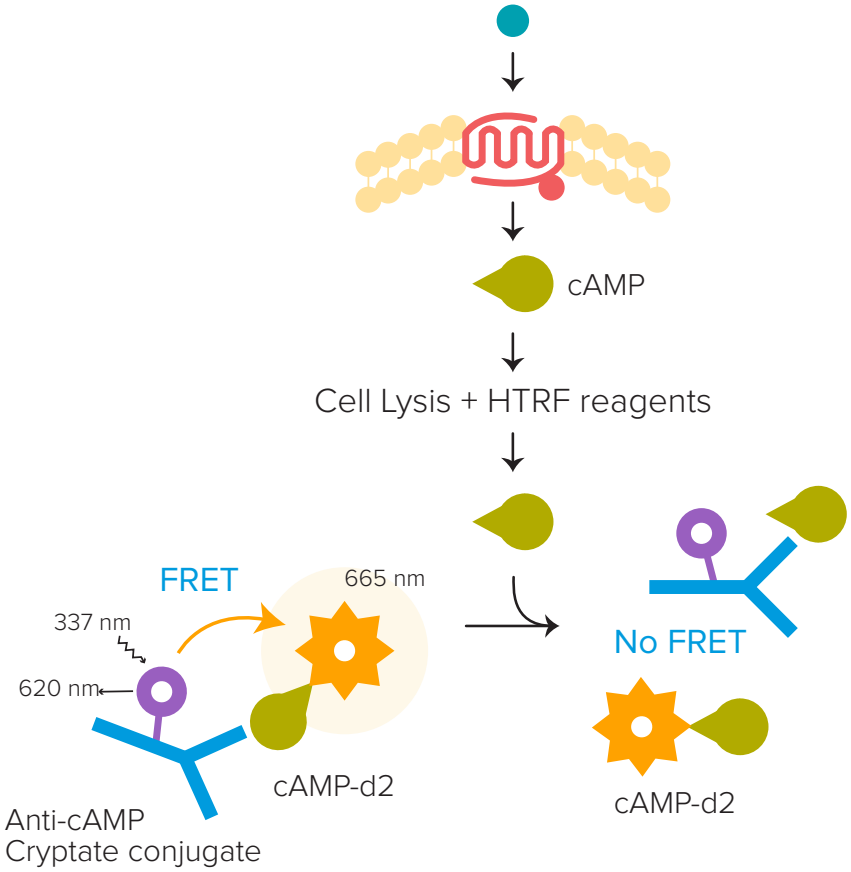

cAMP assay principle.

HTRF cAMP Dynamic 2 and IP-One Assays

The SpectraMax M5e Multi-Mode Microplate Reader with HTRF certification expands the range of assay methods available to researchers who want the flexibility of a dual monochromator-based system and the versatility of multiple detection modes. The IP-One and cAMP HTRF assays from Cisbio, when run on the SpectraMax M5e reader, exhibit wide dynamic ranges and excellent Z' factors ranging from 0.78 to 0.91. Data analysis is simplified using SoftMax Pro Software with preconfigured HTRF protocols.

Benefits

- Flexibility of a dual monochromator-based system
- Wide dynamic ranges and excellent Z' factors
- Preconfigured HTRF protocols with SoftMax Pro Software

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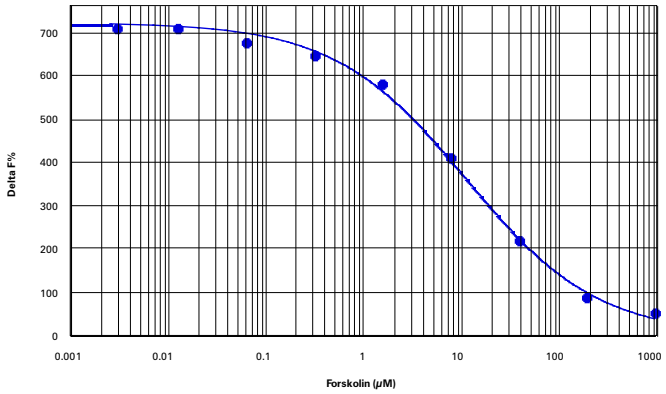
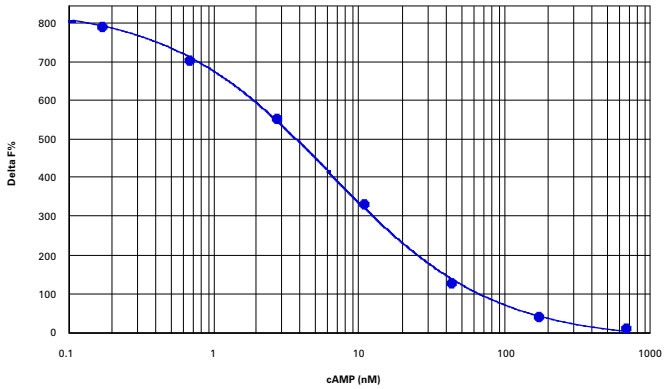
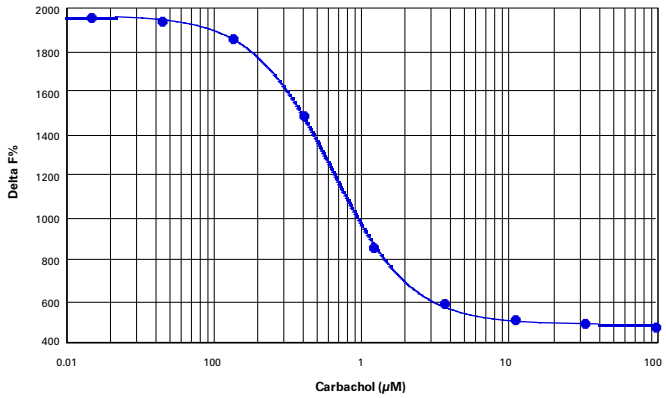


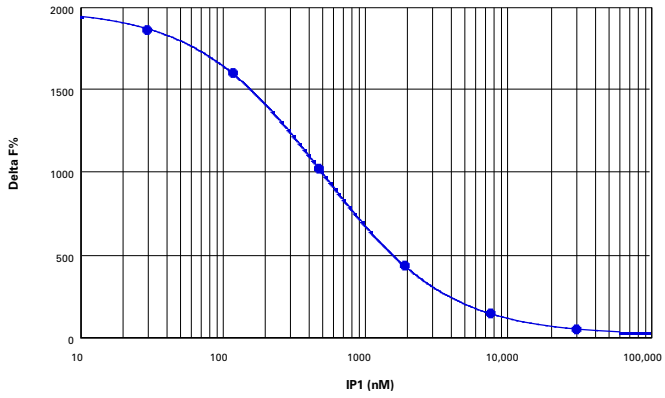
cAMP cell-based assay. Forskolin dose-response in CHO-M1 cells (Z' = 0.86, EC_{50} = 11.4 µM).



cAMP standard curve. cAMP standard curve (Z' = 0.84, EC_{50} = 6.3 nM, comparable to the value indicated in the package insert).



IP1 cell-based assay. Carbachol dose-response curve in CHO-M1 cells (Z' = 0.91, EC_{50} = 0.64 µM carbachol).



IP1 standard curve. Z' = 0.84, EC_{50} =501 nM, comparable to the value indicated in the package insert.

Quant-iT PicoGreen dsDNA assay

Double-stranded DNA is typically quantitated in microplate readers by measuring the absorbance of the DNA solution at 260 nm. However, this method is only able to measure down to about 250 ng/mL on a typical absorbance microplate reader. For biological applications involving small samples, such as purification of DNA fragments for subcloning and quantitation of DNA amplification products, more sensitive methods are needed. The Quant-iT PicoGreen dsDNA Assay from Molecular Probes is more specific for DNA and is about 1000 times more sensitive than traditional absorbance methods. The dynamic range of this assay in microplate format is from 250 pg/mL to 1000 ng/mL with a single dye concentration.

This application note demonstrates that with SpectraMax fluorescence microplate readers and the Quant-iT PicoGreen assay, users can reliably measure down to at least 100 pg/mL of double-stranded DNA.

Benefits

- Sensitive fluorescent quantitation of DNA down to 63 pg/mL
- Linear dynamic range spanning four orders of magnitude
- Easy analysis of results with preconfigured protocol in SoftMax Pro Software

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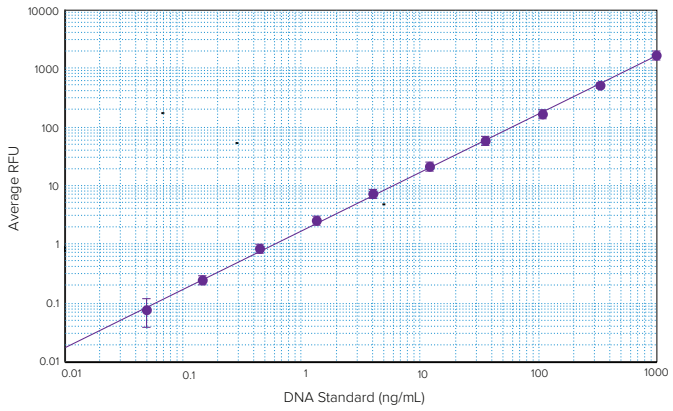
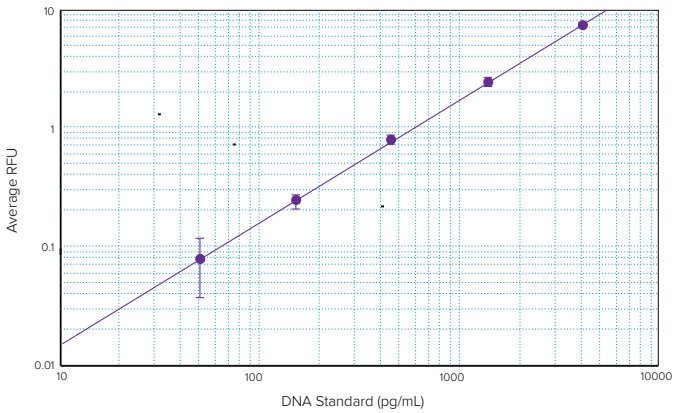


DNA standard curve. DNA standards ranging from 1000 ng/mL to 50 pg/mL were assayed using the Quant-iT PicoGreen dsDNA Assay and SpectraMax M5 microplate reader. The standard curve was plotted using a log-log curve fit in the SoftMax Pro Software (r^2 = 1.000).



Lower end of DNA standard curve. Plot of the lower end of the DNA standard curve, ranging from 4.1 ng/mL to 50 pg/mL (r^2 = 1.000).

Using NanoOrange Protein Kit

Traditional photometer methods, such as absorbance 280, BCA, Bradford or Lowry assays, are not very sensitive in microplate format. The dynamic range of NanoOrange® Protein Quantitation Kit from Life Technologies in microplate format is 10 ng/mL to 10 µg/mL. The data presented in this application note confirms the dynamic range and lower detection limit.

The NanoOrange assay, when run on a Gemini XPS Microplate Reader or other SpectraMax fluorescence microplate reader with SoftMax Pro Software, is a quick, sensitive detection method for proteins. The analysis capabilities of SoftMax Pro Software provide quantitation in an easy-to-read, user-customizable report format.

Benefits

- Greatly improved sensitivity over absorbance methods
- Wide dynamic range from 10 ng/mL to 10 µg/mL
- Easy data acquisition and analysis with preconfigured SoftMax Pro Software protocol

Download Application Note

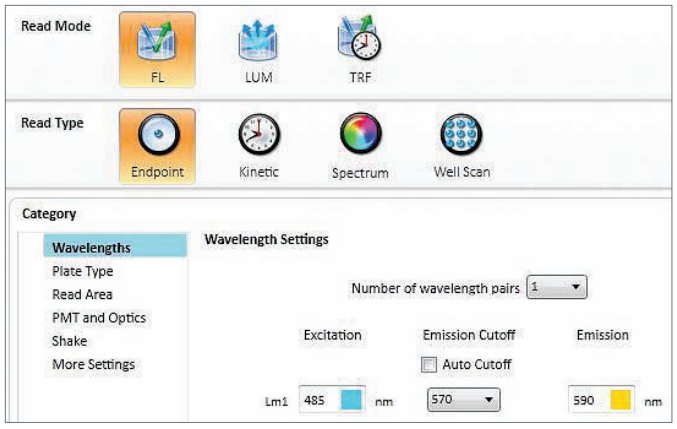
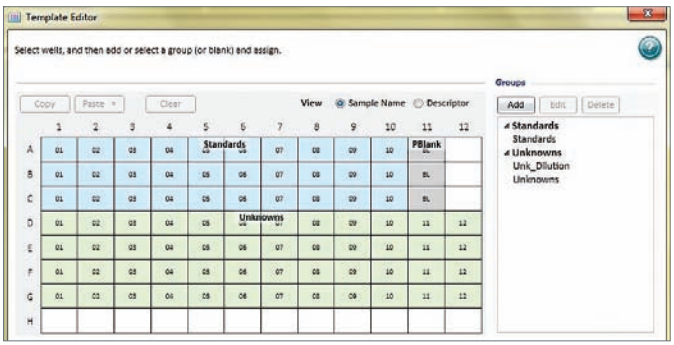
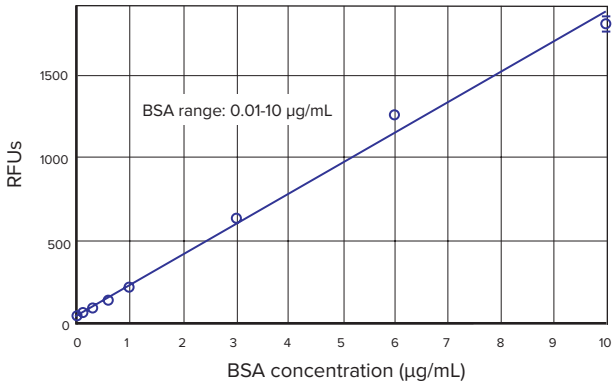


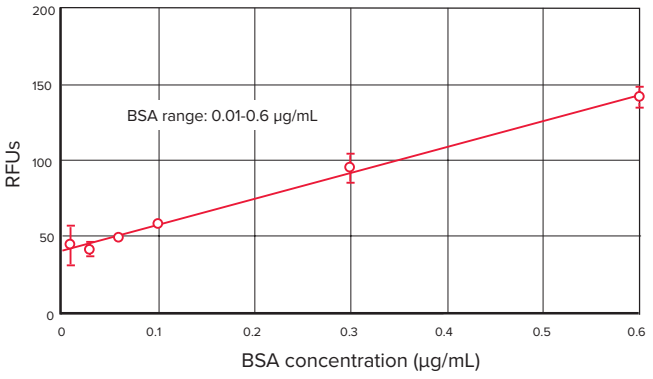
Plate reader settings for NanoOrange assay. Typical settings for SpectraMax readers with fluorescence detection mode are shown.



Template setup. Assigning wells to 'Standards' and 'Unknowns' groups in the Template Editor enables automatic plotting of the standard curve as well as data analysis.

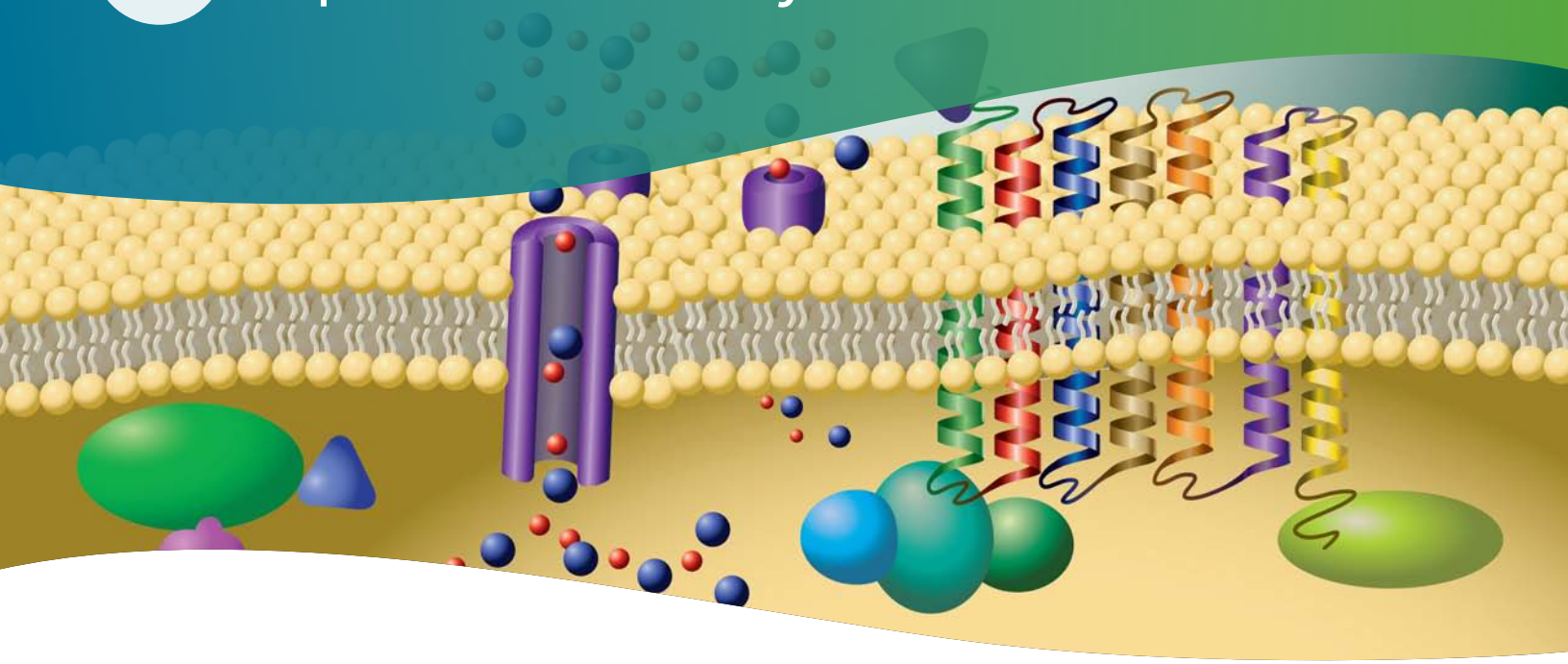


NanoOrange standard curve. Standard curve obtained using BSA standards and a linear curve fit.



Standard curve low end detail. Detail of the lower end of the standard curve shown in figure to the left.

3 Optimized assays



For detailed information, select the image or text.

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- Live Cell

Enzyme Activity

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9/23 2084B
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