

TRANSCREENER® ADP² ASSAYS

Fluorescence-based immunodetection of ADP is the most direct, sensitive, and versatile method for detecting enzymes that convert ATP to ADP.

**ONE ASSAY.
HUNDREDS OF TARGETS.**

Kinases

protein (tyrosine, serine/threonine)
lipid (sphingosine kinase, PI3K (α , β , Δ and γ))
carbohydrate (ketohexokinase, hexokinase
phosphofructokinase)

Other ATP Utilizing Enzymes

heat shock proteins, acetyl CoA carboxylase, glutamine synthetase, ATP citrate lyase, RecA, helicase

- **DIRECT ADP DETECTION**

Fewer reagents and fewer steps means less chance for compound interference, less secondary screening, and less chance of missing a hit than with indirect methods.

- **THREE DETECTION MODES**

FP, TR-FRET and FI detection validated on all the major multimode plate readers. No matter what instrument you use, you'll know which filters and settings to use for optimal performance.

- **SENSITIVE**

Easily detects less than 10% ATP conversion using 0.1 to 1000 μ M ATP, assuring accurate inhibitor potencies and minimizing enzyme and substrate costs.

- **PROVEN PERFORMANCE**

Used in over 40M wells of pharma screening since 2006, Transcreener is the most extensively validated ADP detection method available backed by the best technical support in the industry.

ADP detection

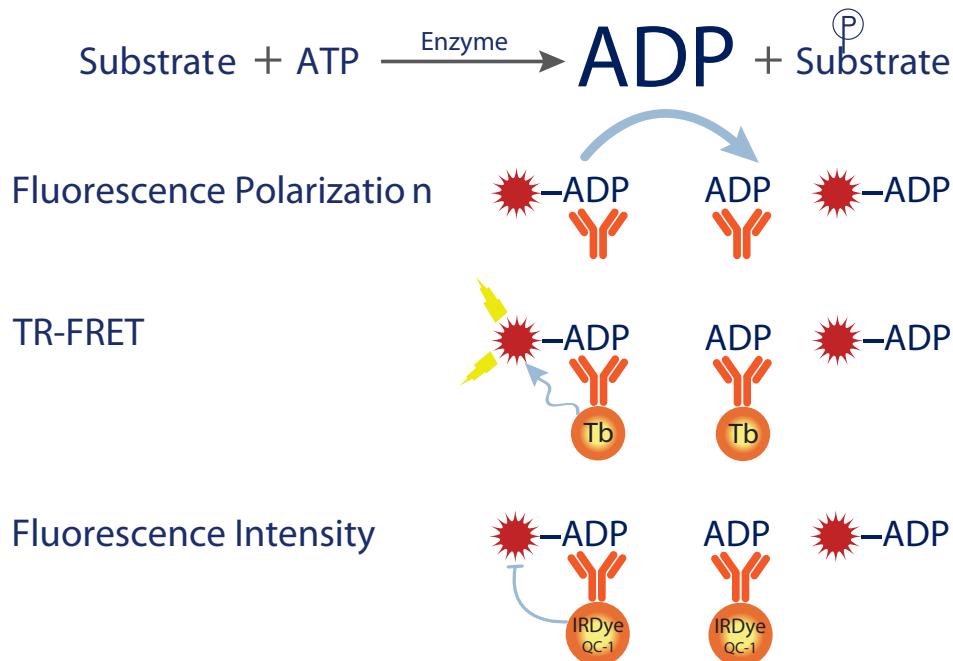
TRANSCREENER®
High Throughput Screening Assays



TRANSCREENER® ADP² ASSAYS

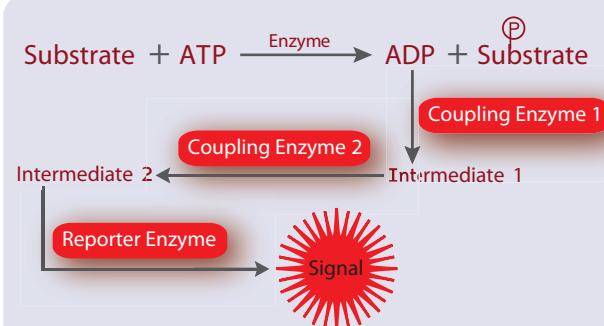
Direct ADP detection.

Transcreener is the simplest, most direct ADP detection method available: binding of ADP to antibody displaces a tracer, causing a change in its fluorescence properties [1]. It has fewer reagents, fewer assay steps and less chance of interference compared with other methods, all of which rely on coupling and/or reporter enzymes.



Your choice of fluorescent detection modes.

There are a number of reasons for choosing one detection mode over another: minimizing compound interference, maximizing instrument performance, comparison to previous data, or just familiarity and comfort level. We've made it possible to use Transcreener ADP² Assays with the three most common fluorescent detection modes: fluorescence polarization (FP), time resolved fluorescence resonance energy transfer (TR-FRET) and fluorescence intensity (FI). All three assays use far red fluors, which ensures that interference from fluorescent library compounds and light scattering is reduced to insignificant levels [1, 2].



Other ADP or ATP detection methods use coupling enzymes to convert ADP to a product that can be detected as well as reporter enzymes, to generate a signal. Each coupling and reporter enzyme is a potential target for the compounds being screened (3), which increases the risk of false positives or of missing a hit. Some coupled methods require extra liquid addition and incubation steps, which complicates assay automation.

1. Vedvik, K.L., et al., Assay Drug Dev Technol, 2004. 2(2): p. 193-203.

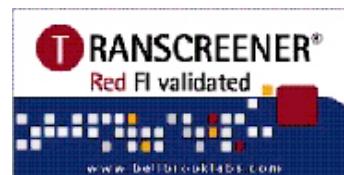
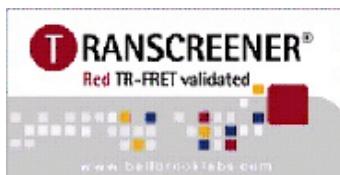
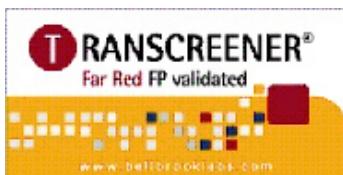
2. Simeonov, A., et al., J Med Chem, 2008. 51(8): p. 2363-2571.

3. Auld, D.S., et al., J Med Chem, 2008. 51(8): p. 2372-2386.

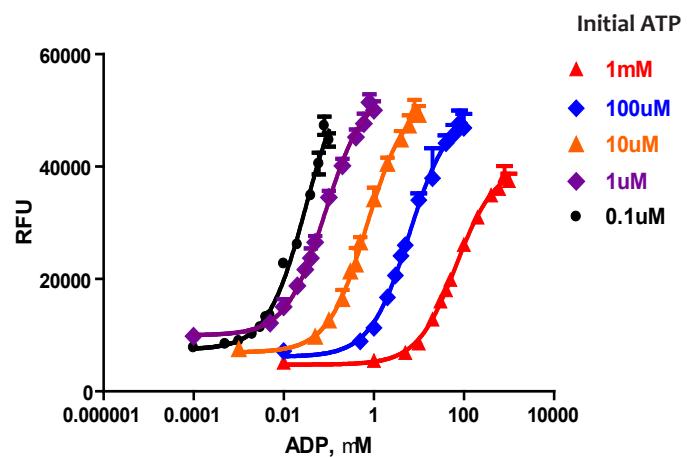
TRANSCREENER® ADP² ASSAYS

Validated performance on major instrument platforms.

In addition to giving you flexibility of three detection modes, we have collaborated with the major suppliers of multimode readers to optimize instrument hardware and software settings for maximal performance with each of the Transcreener ADP² Assays. Successful validation requires a Z' of greater than 0.7 for ADP detection under initial velocity conditions ($\leq 10\%$ conversion) using initial ATP concentrations from 0.1 to 1,000 μM . This ensures that whatever fluorescent detection mode you prefer, you will get robust results with Transcreener reagents.



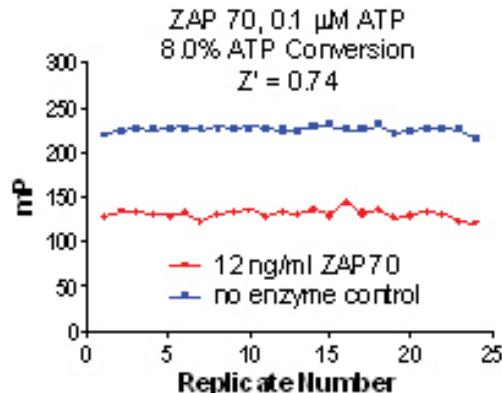
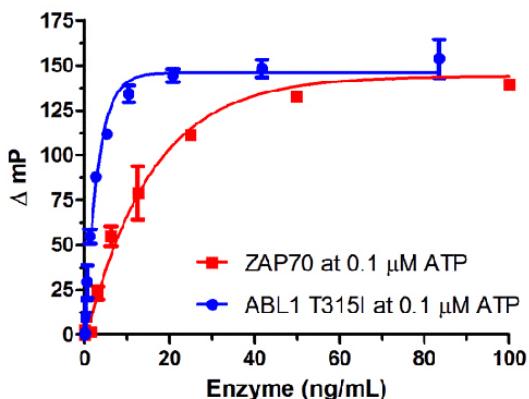
Instrument Partner	Plate Reader
TECAN.	Safire TM , Infinite [®] M200, Infinite [®] F200, Infinite [®] F500, Infinite [®] M1000, Infinite [®] 200 PRO
PerkinElmer[™] instruments.	Envision [®]
BMG LABTECH	PHERAstar FS, PHERAstar Plus, FLUOstar Omega, POLARstar Omega, FLUOstar Optima, POLARstar Optima
Molecular Devices	Analyst [®] GT, SpectraMax [®] M2, SpectraMax [®] M3, M4, M5 and M5e, Gemini, FlexStation 3, SpectraMax Gemini XPS/EM
BioTek	Synergy TM H4, Synergy TM 2, Synergy TM 4

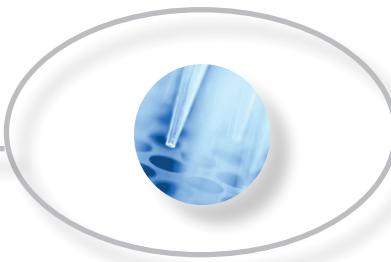


ATP/ADP standard curves that mimic the conversion of ATP to ADP in an enzyme reaction are part of the validation of the ADP² FI Assay with the Perkin Elmer Envision.

The most sensitive ADP detection method available.

Because the antibody has very high affinity and selectivity for ADP vs. ATP, the assays are capable of detecting very low quantities of ADP – less than 10nM – in the presence of a 10-100-fold excess of ATP. And this is achieved over a broad range of initial ATP concentrations - from 100nM to 1mM - with Z' values of greater than 0.7. This means you can detect enzyme initial velocity using ATP concentrations at or below the Km for your enzyme, giving you accurate inhibitor potencies with better data quality and less enzyme compared with other methods.





biology at work

TransCREENER® GDP ASSAYS

GTPases

Fucosyltransferase

Mannosyltransferase

TRANSCREENER PROVIDES EFFICIENCY.

Screen GTPases, fucosyltransferase or mannosyltransferase. Covers a broad range of GTP concentrations. Use from assay development to HTS.

TRANSCREENER IS ECONOMICAL.

Use less enzyme and get excellent Z' values at a low GTP % conversion

TRANSCREENER ENSURES COMPATIBILITY.

Two detection modes for compatibility with more instrument platforms.

GDP detection

TWO DETECTION MODES

Now available in 2 detection modes in the red emission spectrum to minimize compound interference. The result is compatibility with more instrument platforms.

- Fluorescence Polarization
- Fluorescence Intensity

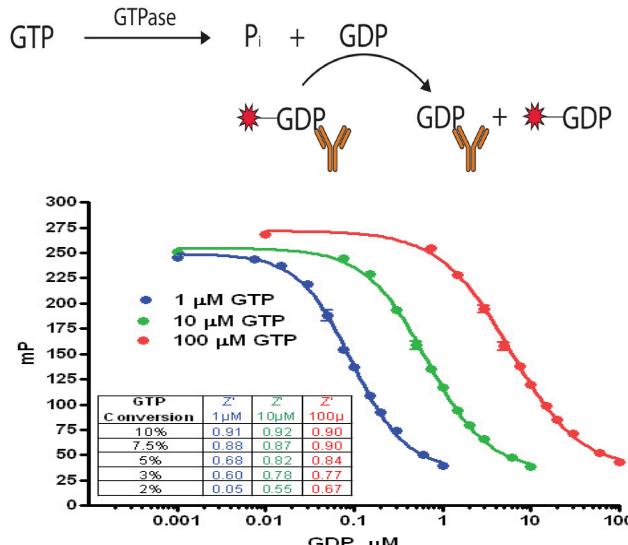
TRANSCREENER®
High Throughput Screening Assays



TRANSCREENER® GDP FP ASSAY

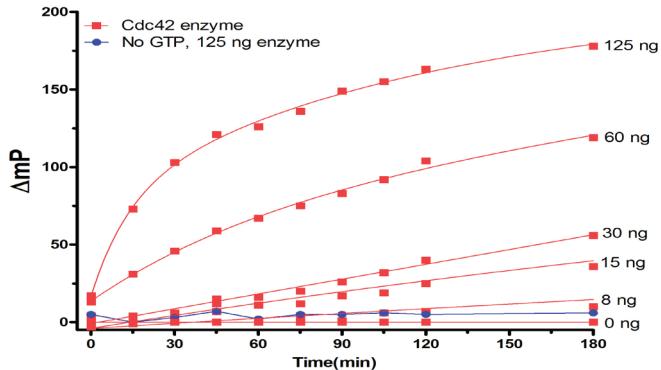
Transcreener® GDP FP Assay

far red FP



To mimic GDP generated during a GTPase reaction, standard curves were prepared over a wide range of GTP concentrations, keeping the guanosine concentration constant. Excellent Z' values were obtained at less than 5% GTP conversion.

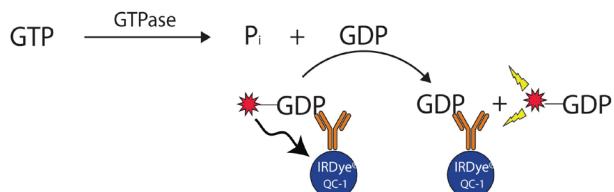
The Transcreener GDP FP Assay was developed to follow the progress of any enzyme that produces GDP. The Transcreener GDP Detection Mixture comprises a GDP Alexa633 Tracer bound to a GDP Antibody. The tracer is displaced by GDP, the invariant product generated during the enzyme reaction. The displaced tracer freely rotates leading to a decrease in fluorescence polarization.



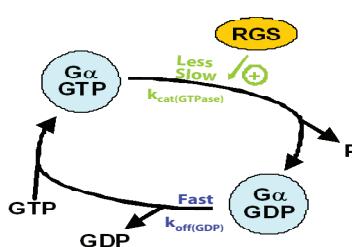
Time-dependent GDP production with varying CDC42 enzyme concentrations. The small GTPase was incubated with 10 μM GTP in a total volume of 20 μL.

TRANSCREENER® GDP FI ASSAY

Transcreener® GDP FI Assay

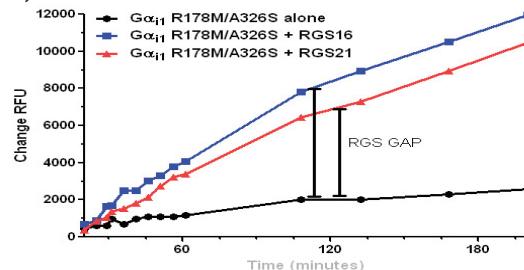


A) RGS GAP Activity Schematic



Gαi1 R178M/A326 allows measurable RGS GAP activity by measuring GDP. A) Gαi1 R178M/A326S was engineered as a model protein to detect RGS GAP activity in a steady state assay by altering the Gαi1 GDP dissociation rate, which is the rate limiting step, and the intrinsic GTPase rate. B) 50 nM Gαi1 R178M/A326S was added to a reaction mix with 250 nM RGS16, RGS21 or without in a 384 well low volume assay plate and incubated at 30°C. The plate was read at various time points. N = 16. Change RFU = RFUGα(+/- RGS) - RFU no Gα(+/- RGS). This indicates that both RGS isoforms are GAPs for Gαi1 R178M/A326S.

B) RGS Isoforms



Cat#	Product	Size
3009-1K	Transcreener GDP FP Assay	approx. 1,000 assays
3014-1K	Transcreener GDP FI Assay	approx. 1,000 assays

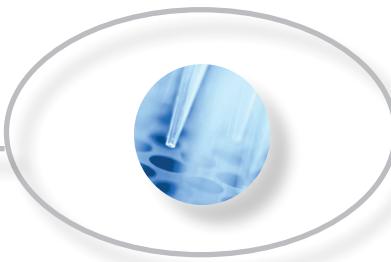
For orders of 10,000 wells or more please contact us for a price quote.

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Transcreener is a registered trademark of BellBrook Labs. Transcreener is a patented technology of BellBrook Labs. AlexaFluor® is a registered trademark of Molecular Probes, Inc (Invitrogen). IRDye® QC-1 is supplied through a licencing agreement with LI-COR Inc. IRDye® is a registered trademark of LI-COR Inc.

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Labs

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biology at work

TransCREENER[®] AMP/GMP ASSAY

LIGASES

TRANSCREENER PROVIDES EFFICIENCY.

Single assay for 1 to 1,000 μ M ATP

Any AMP/GMP producing enzyme. Any Substrate.

PHOSPHODIESTERASES

SYNTHETASES

TRANSCREENER IS ROBUST

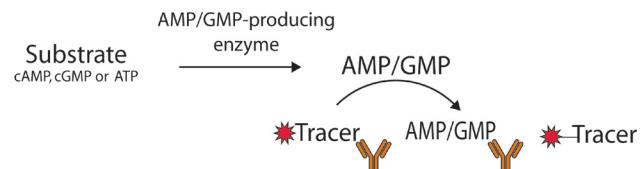
Ratiometric, red-shifted fluorescence polarization output minimizes signal variability and reduces compound interference, resulting in robust Z' values ≥ 0.7 .

AMP/GMP detection

FAR RED FLUORESCENCE POLARIZATION

Transcreener[®] AMP/GMP Assay

far red FP

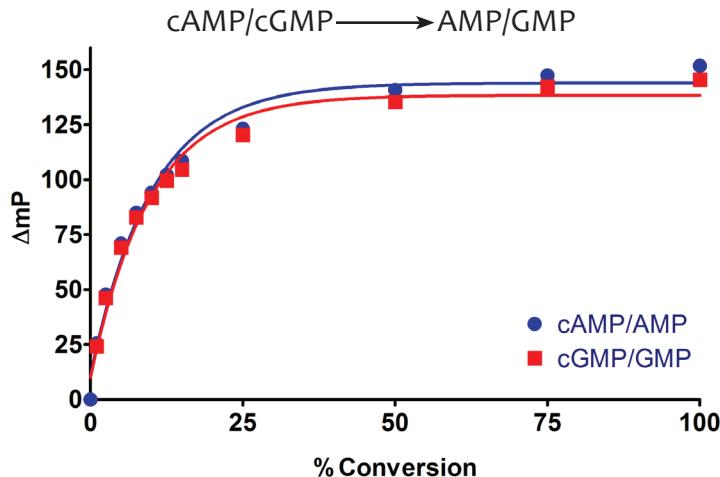


The Transcreener AMP/GMP Assay was developed to follow the progress of any enzyme that produces AMP or GMP. Enzyme reaction progress is indicated by a decrease in the fluorescence polarization. The Transcreener AMP/GMP Detection Mixture comprises an AMP/GMP Alexa633 Tracer bound to an AMP/GMP Antibody. The tracer is displaced by AMP or GMP, the invariant product generated during the enzyme reaction. The displaced tracer freely rotates leading to a decrease in polarization.

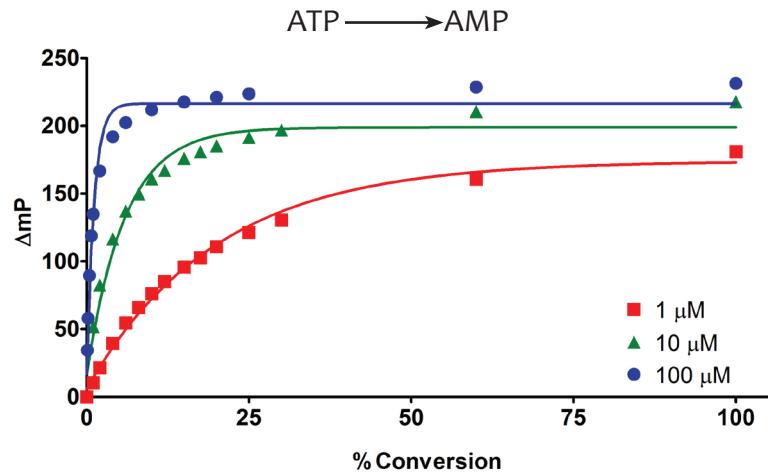
TRANSCREENER® AMP/GMP ASSAY

EXCELLENT DATA QUALITY

The Transcreener AMP/GMP Assay is sensitive down to 1 μ M initial concentrations of cAMP, cGMP or ATP and provides excellent Z' values at low % conversion.

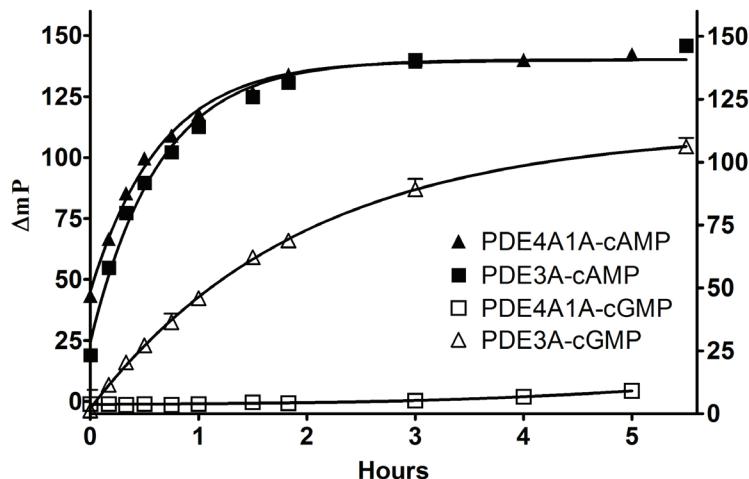


Substrate standard curve demonstrating robust signal at low percent conversion with 1 μ M cGMP or cAMP substrates.

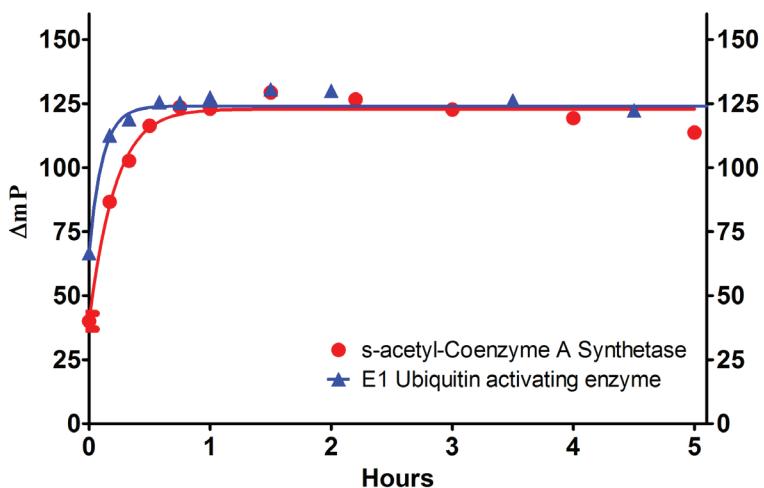


Substrate standard curve demonstrating robust signal at low percent conversion with three different concentrations of ATP.

ANY AMP OR GMP-PRODUCING ENZYME. ANY SUBSTRATE.



Substrate specificity for 2 PDE enzymes using both cGMP and cAMP substrates.



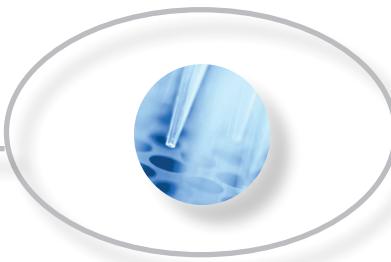
Enzyme assays run using two ATP-utilizing enzymes and 1 μ M ATP substrate.

Cat#	Product	Size
3006-1K	Transcreener AMP/GMP Assay	approx. 1,000 assays

For orders of 10,000 wells or more please contact us for a price quote.



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biology at work

TransCreener[®] UDP ASSAY

UDP-GLYCOSYLTRANSFERASES

glucosyltransferase, galactosyltransferase,
glucuronyltransferase,
N-acetylglucosamyltransferase,
N-acetylgalactosyltransferase, xylosyltransferases

SYNTHASES

glycogen, cellulose, lactose and hyaluronan

TRANSCREENER PROVIDES EFFICIENCY.

Single assay for 1.0 to 1,000 μM UDP-Sugar
Any UDP-producing enzyme. Any Substrate.

TRANSCREENER IS ROBUST.

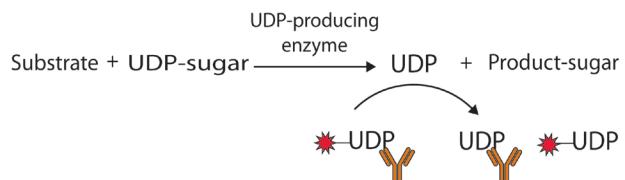
Ratiometric, red-shifted fluorescence polarization output minimizes signal variability and reduces compound interference, resulting in robust Z' values $\geq 0.7 ..$

UDP detection

FAR RED FLUORESCENCE POLARIZATION

Transcreener[®] UDP Assay

far red FP



The Transcreener UDP Assay was developed to follow the progress of any enzyme that produces UDP. The Transcreener UDP Detection Mixture comprises an UDP DyLight™632 Tracer bound to an UDP Antibody. The tracer is displaced by UDP, the invariant product generated during the enzyme reaction. The displaced tracer freely rotates leading to a decrease in fluorescence polarization. Therefore, UDP production leads to a decrease in polarization.

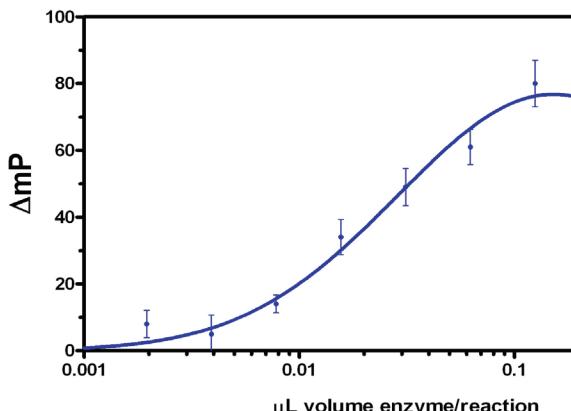
TRANSCREENER® UDP ASSAY

There are over 200 glycosyltransferases encoded in the human genome, and most use uridine diphosphate (UDP)-activated sugars as the donor. From a functional standpoint, the reactions they catalyze can be divided into three major types: biosynthesis of disaccharides or polymers such as starch or hyaluronan, posttranslational modification of proteins, and glucuronidation of small molecules, including endogenous hormones and xenobiotics. All three types of glycosyltransferases are of interest in drug discovery.

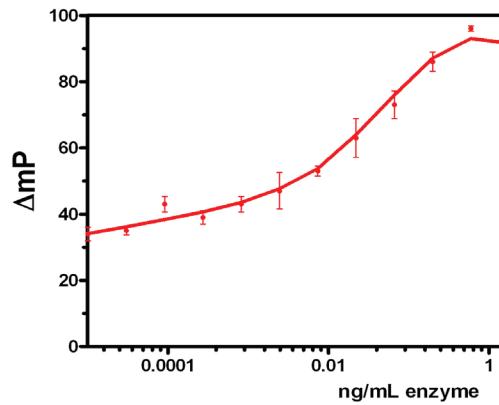
The Transcreener UDP Assay has been designed to provide Z' values routinely greater than 0.7 in 384-well, 20 μ L reaction format. This assay has demonstrated great flexibility for detecting a range of UDP-producing enzymes, and donor- and acceptor-substrate concentrations.

SURVEY DIVERSE ENZYME FAMILIES WITH ONE SET OF REAGENTS

A) Glucosylceramide Synthase Titration



B) α -1,3-Galactosyltransferase Titration



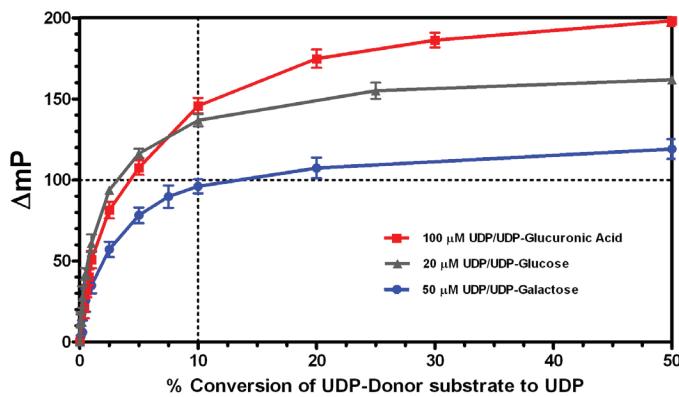
A) Enzyme dependent UDP production was demonstrated with Glucosylceramide Synthase utilizing the Transcreener UDP Assay kit.

15 μ L reactions were performed in assay conditions of 50 mM HEPES pH 7.5, 5 mM MnCl₂, 20 mM KCl, 80 mM EGTA, 1% DMSO, 20 μ M UDP-Glucose, +/- 5 μ M C-8 Ceramide, 35 μ g/mL UDP antibody, and 2 nM UDP DyLight™ 632 Tracer. Polarization (mP) was measured with a Tecan Safire™. The data for this plot was generated after 2 hours at 37°C. ΔmP data was generated by subtracting mP data from reactions containing C-8 ceramide from mP data from reactions without C-8 ceramide.

B) α -1,3-Galactose Transferase titration using the Transcreener UDP Assay.

15 μ L reactions were performed in assay conditions of 10 mM Tris pH 7.0, 10 mM MnCl₂, 50 μ M UDP-Galactose, +/- 50 mM lactose, 35 μ g/mL UDP antibody, and 2 nM UDP DyLight™ 632 Tracer. Polarization (mP) was measured with a Tecan Safire™. The data for this plot was generated after 2 hours at 37°C.

USE ANY UDP-SUGAR DONOR SUBSTRATE



Standard curve data plotted from an 20 μ M UDP/UDP-Glucose, 50 μ M UDP/UDP-Galactose and 100 μ M UDP/UDP-Glucuronic Acid standard curves. This mimics a typical enzyme reaction (as UDP is produced, UDP-donor is depleted) by keeping the total nucleotide concentration constant. This data shows the flexibility of the Transcreener UDP Assay using different donor-substrates and different enzyme reaction conditions.

Cat#	Product	Size
3007-1K	Transcreener UDP Assay	approx. 1,000 assays

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TRANSCREENER® ADP² ASSAYS

Transcreener® Literature References

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Expert Opin Ther Targets 2006;10(1):179-190 Transcreener™: screening enzymes involved in covalent Regulation. Robert G Lowery &

Ordering Information

Cat#	Product	Size
3010-1K	Transcreener ADP ² FP Assay	approx. 1,000 assays (384 well)
3011-1K	Transcreener ADP ² TR-FRET Red Assay	approx. 1,000 assays (384 well)
3013-A	Transcreener ADP ² FI Assay	approx. 200 assays (96 well)
3013-1K	Transcreener ADP ² FI Assay	approx. 1,000 assays (384 well)

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