BellBrook Labs

BIOLOGY AT WORK

Assay Tools for HTS and Lead Discovery

Product Catalog

Transcreener® ADP² Assay

Enzyme Targets: Protein Kinases, Lipid Kinases, Carbohydrate Kinases, ATPases, Chaperonins (HSP90, etc.), Nucleotidases, Carboxylases, Helicases & More!

Detection Formats: FP, FI, or TR-FRET

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 fluorescent properties. It has fewer reagents, fewer assay steps, and less chance of interference compared with other methods, all of which rely on coupling and reporter enzymes.

Most Sensitive ADP Detection Method Available

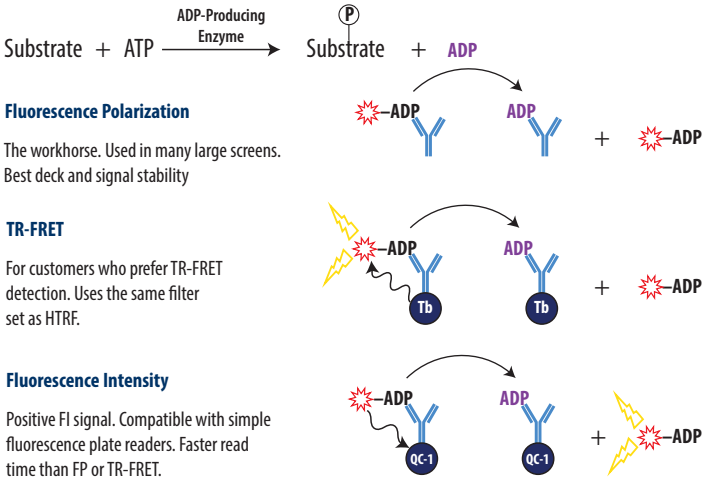
High affinity, ultra-selective ADP antibody detects less than 10 nM ADP, meaning low enzyme usage and high Z' values.

Validated on Major Multi-Mode Readers

We have collaborated with major suppliers of multi-mode readers to optimize instrument hardware and software settings for maximal performance with each detection mode. And just as important, the assays have been used successfully in millions of wells with a diverse mix of targets including protein, lipid, and carbohydrate kinases, chaperonins, carboxylases, helicases, and nucleotidases.

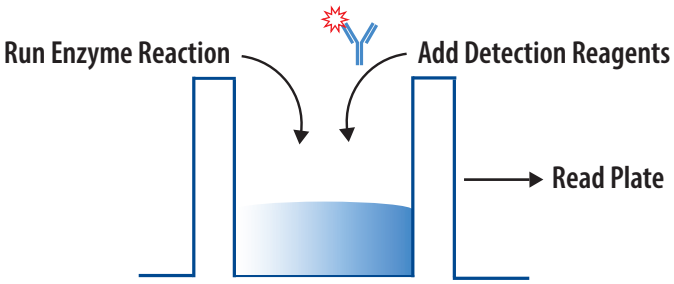
Choice of Fluorescence Detection Modes

FP, FI, and TR-FRET detection validated on all major multi-mode readers. No matter what instrument you choose you'll know which filters and settings to use for optimal results.



Single Addition, Mix-and-Read Format for Easy Automation

Run your enzyme reaction, add detection reagents, and read. It's that simple.

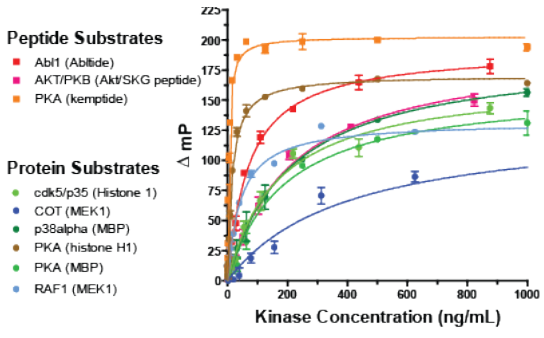


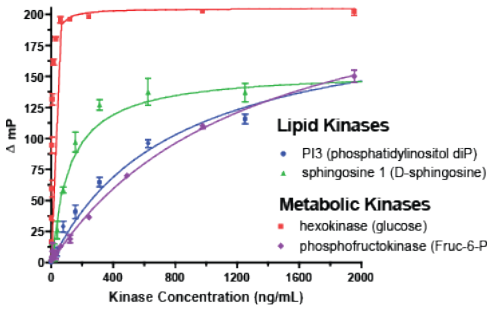
>12 Hour Reagent and Signal Stability

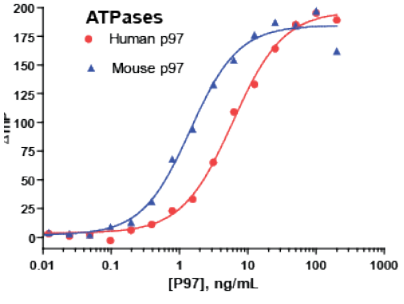
No other ADP assay method has this level of stability. Provides flexibility to fit work-flows in automated HTS environments.

Universal: Use Any Substrate or ATP Concentration

Complete flexibility for peptide, protein, or small molecule substrates and can accommodate any ATP concentration from 100 nM to 1 mM. Allows detection of protein, lipid, or carbohydrate kinase activity at a variety of ATP concentrations.







Transcreener® cGAMP cGAS Assay

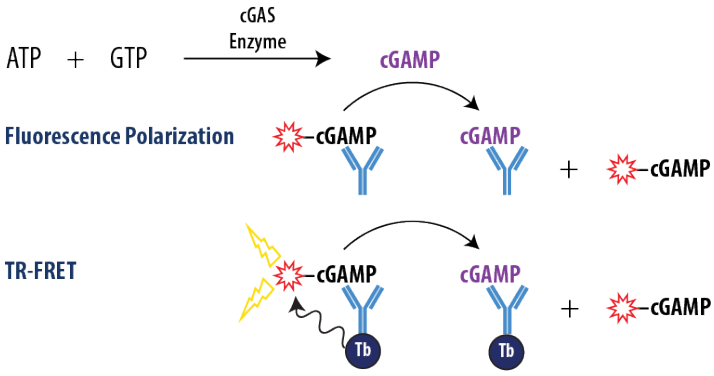
Enzyme Target: Cyclic AMP-GMP Synthase (cGAS)

Detection Formats: FP, TR-FRET

Discover cGAS Inhibitors with a Simple Biochemical Assay

The Transcreener cGAS Assay directly measures cyclic GMP-AMP (cGAMP) produced by cyclic GMP-AMP synthase (cGAS). By measuring the production of cGAMP, researchers can effectively determine activity of the cGAS enzyme. The assay provides a powerful tool to screen entire compound libraries for cGAS modulators to help find new therapeutics targeting the cGAS-STING pathway.

Direct Detection of cGAMP with an FP or TR-FRET Readout

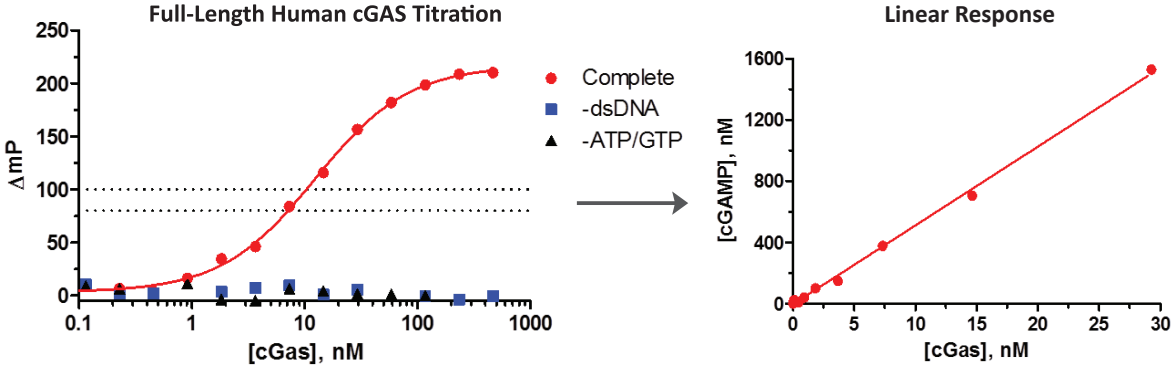


Simple, Mix-and-Read, HTS-Ready

The Transcreener cGAS Assay is in a simple, HTS-ready, mix-and-read format. Run your enzyme reaction, add Transcreener reagents, and read your plates. The assay is compatible with 96, 384, and 1536-well formats.

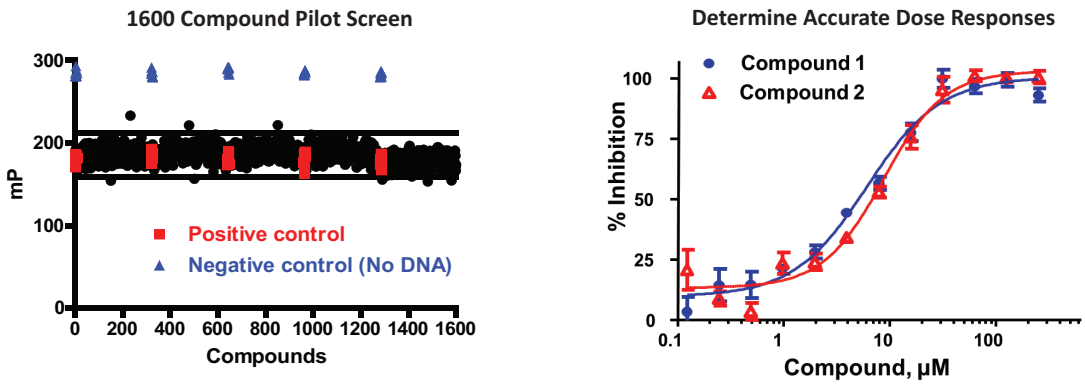
Detection of cGAMP Under cGAS Initial Velocity

The assay demonstrates linearity when raw data is converted to cGAMP using a standard curve. cGAS is dependent on double-stranded DNA as well as ATP and GTP substrates for the production of cGAMP.



Pilot Screen & Dose-Response

The Transcreener cGAS Assay is designed for screening compound libraries in a high throughput format. Follow up SAR can also be performed using the assay to determine inhibitor potency with ease.



Transcreener® GDP Assay

Enzyme Targets: Any GTPase: Gα proteins, Ras-like G proteins (Ras, Roc, Rac, Cdc42, Rab, etc.) GAPs, GEFs, Fucosyltransferases

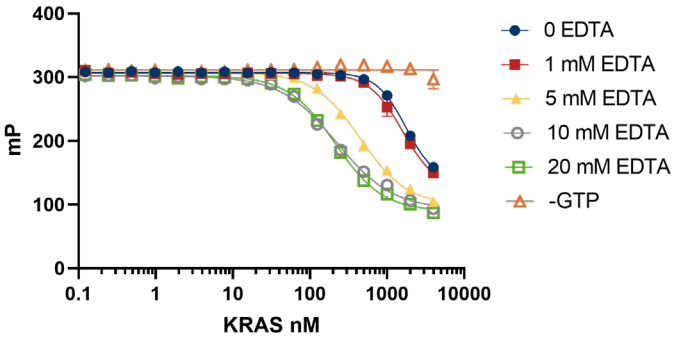
Detection Formats: FP, FI, or TR-FRET

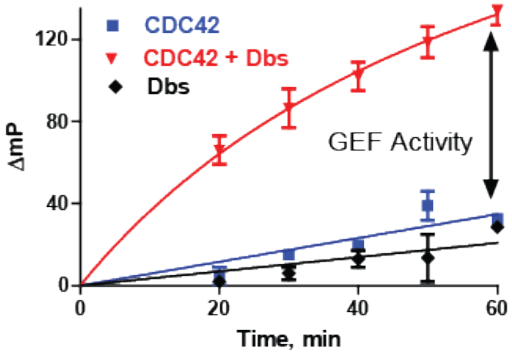
Mix-and-Read Fluorescent GTPase Assay

The Transcreener GDP GTPase assay is a homogenous, fluorescent immunoassay that monitors the activity of GTPases along with GEF and GAP partners. The assay directly measures the amount of GDP produced in an *in vitro* GTPase reaction.

Assay Features

- Easy-to-Use, ultra-sensitive GTPase activity assay
- Tunable dynamic range to match the target of interest
- HTS Compatible with 96, 384, and 1536-well plates
- Direct detection of GDP to monitor GTPase, GAP, and GEF Activity
- Stable assay reagents – minimum 8-hour reagent and signal stability even at room temperature!





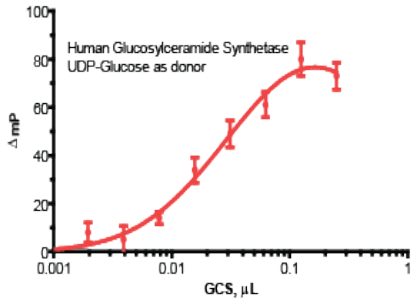
Transcreener® UDP² Assay

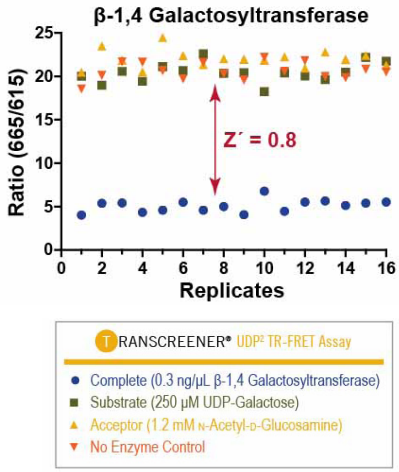
Enzyme Targets: Glycosyltransferases (Glucosyltransferase, Glucuronosyltransferases, Galactosyltransferase, etc.), Glycogen Synthetase

Detection Formats: FP, FI, or TR-FRET

Fluorescent Glycosyltransferase Assay

The Transcreener UDP² Glycosyltransferase Assay is a single step, homogenous, fluorescent assay for detection and screening of UDP-producing enzymes. Direct detection of UDP with an FP, FI, or TR-FRET readout provides a safe, HTS-compatible alternative to cumbersome radioassay methods and is more sensitive and less subject to interference than coupled assays, where the UDP is converted to another product. Transcreener is compatible with any enzyme class that produces UDP, including UDP-glucose-, UDP-galactose- and UDP-glucuronosyltransferases as well as glycogen, hyaluranon, and cellulose synthases.





Assays for Methyltransferases

AptaFluor® SAH Methyltransferase Assay

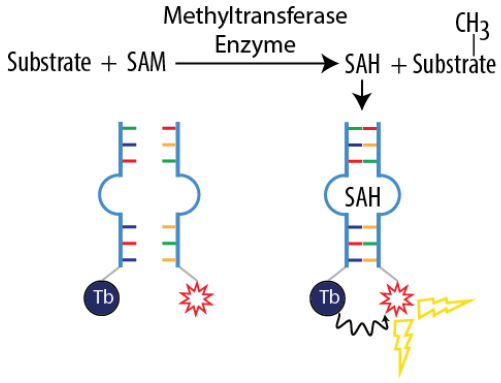
Detection Format: TR-FRET

Leveraging the Power of Aptamers for HTS Assays

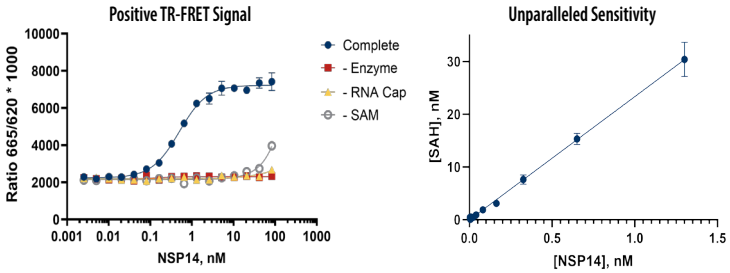
The AptaFluor SAH Methyltransferase Assay uses a naturally occurring aptamer that selectively binds SAH, the invariant product of methyltransferase reactions. The exquisite affinity and selectivity of the aptamer combined with a positive TR-FRET signal enable screening and profiling of methyltransferases with unparalleled sensitivity.

Ultra-Sensitive

The most sensitive HTS methyltransferase assay available with an LLD of 0.6 nM SAH. This dramatically reduces enzyme usage and allows the assay to be run at or below K_m for SAM.



NSP14 Titration with 100 nM SAM



Transcreener® EPIGEN SAH Methyltransferase Assay

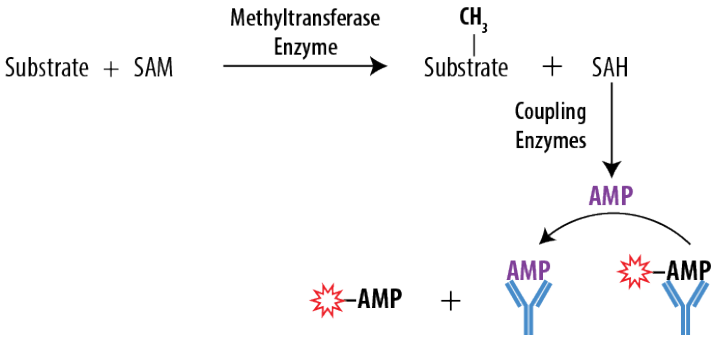
Detection Format: FP

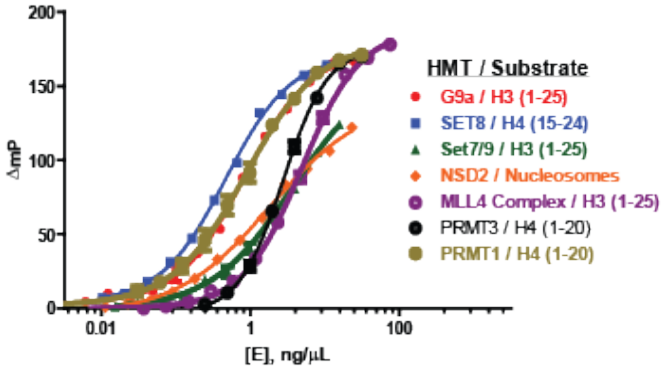
Universal Method for Virtually Any Methyltransferase

The Transcreener EPIGEN SAH Assay provides universal methyltransferase detection in an HTS-proven format. It combines the extensively validated Transcreener AMP²/ GMP² Assay with coupling enzymes that convert the SAH produced in a methyltransferase reaction to AMP for detection with a fluorescent polarization (FP) readout.

Simple & HTS-Ready

The Assay is simple, HTS-ready and has the outstanding data quality and deck and signal stability that Transcreener Assays are known for. Run your enzyme reaction, add the quench reagent, add detection reagents (coupling enzymes are pre-combined with Transcreener reagents), and read your plates.





Transcreener® AMP²/GMP² Assay

Enzyme Targets: Phosphodiesterases, Ub ligase, SUMO ligase, DNA ligase, Acyl CoA synthetase, AA-tRNA synthetase, NAD synthetase, CD39, ENPP1

Detection Formats: FP or TR-FRET

Universal AMP/GMP Detection

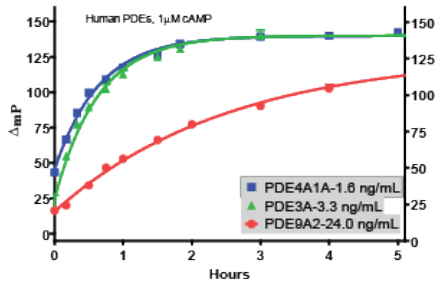
Detect any AMP/GMP producing enzyme (e.g., ligases, synthetases, phosphodiesterases) using any precursor substrate, including cAMP, cGMP, ATP, or NAD.

It is the only activity assay method for direct detection of unlabeled AMP or GMP; i.e. without using coupling enzymes.

The method is simple and HTS-proven: run your enzyme reaction, add detection reagents, and read the far-red FP or TR-FRET signal on any multi-mode plate reader.

Applications

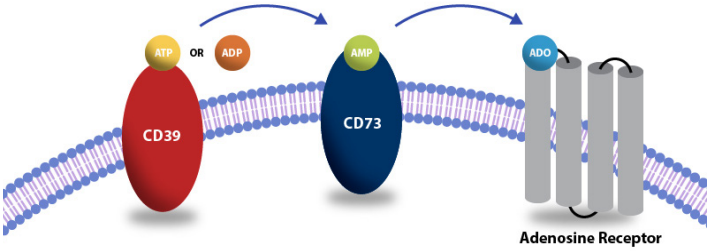
- Measure enzymatic activity
- Screen compound libraries for inhibitors
- Determine inhibitor selectivity
- Measure inhibitor potency
- Inhibitor residence time measurements



Features

- Direct detection of unlabeled AMP or GMP from a PDE reaction
- Easy to use: one-step, simple mix-and-read format
- Robust: Z' > 0.7 for initial velocity reactions
- Non-radioactive assay technique
- Wide substrate concentration range: 1 µM to 1000 µM

Target Focus: CD39

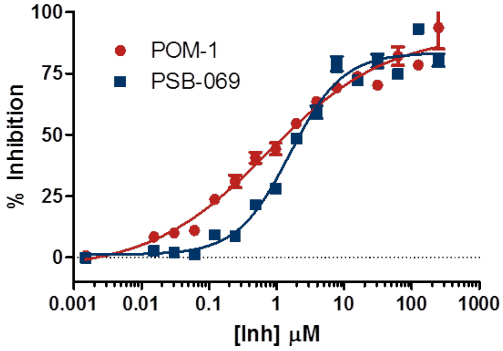


CD39 is an Important Messenger for Purinergic Signaling

Ectonucleotidases are plasma membrane-bound enzymes with externally oriented active sites that metabolize nucleotides to nucleosides and are crucial for maintaining immune homeostasis. CD39, hydrolyzes ATP and ADP to AMP. AMP can further be processed to adenosine leading to a significant impact on many disease states. Recent studies have shown a key role for adenosine in immunosuppression in the tumor microenvironment, and ectonucleotidases are emerging as promising immuno-oncology targets.

Discover CD39 Inhibitors with the Transcreener AMP²/GMP² Assay

The Transcreener AMP²/GMP² Assay directly measures AMP produced by ectonucleoside triphosphate diphosphohydrolase-1 (also known as ENTPD1, NTPDase1, Cluster of Differentiation 39 or CD39). By measuring the production of AMP researchers can effectively determine the activity of the CD39 enzyme. The assay provides a powerful tool to screen entire compound libraries for CD39 modulators to help find new therapeutics for diseases such as cancer.



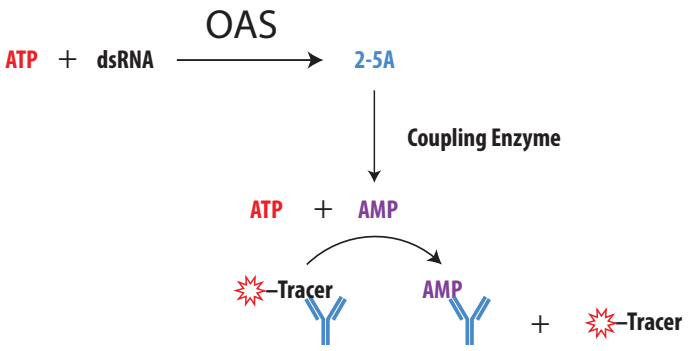
Transcreener® 2-5A OAS Assay

Enzyme Target: OAS1, OAS2, OAS3

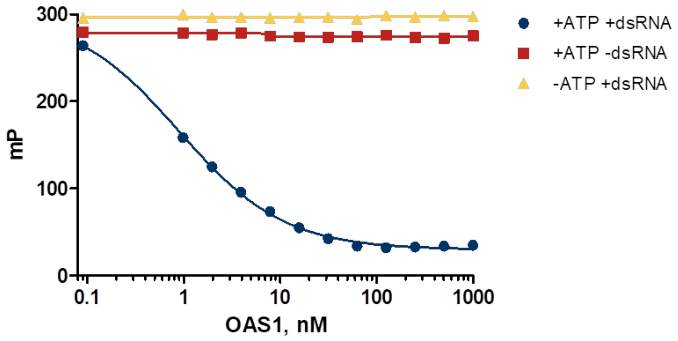
Detection Formats: FP

Discover OAS Inhibitors

The Transcreener OAS Assay measures the 2-5A produced by 2′-5′-oligodenylate synthetase 1. Double stranded RNA enters the cell from viral infection.



OAS1 Enzyme Titration



Features

- Detection of unlabeled 2-5A
- Easy-to-use, homogenous, one-step format
- Robust Assay Z′ > 0.7 under initial velocity conditions

Applications

- Measure enzymatic activity of OAS enzymes
- Screen compound libraries for OAS inhibitors
- Quantify inhibitor potency (IC_{50})
- Inhibitor selectivity profiling

Transcreener® ADO CD73 Assay

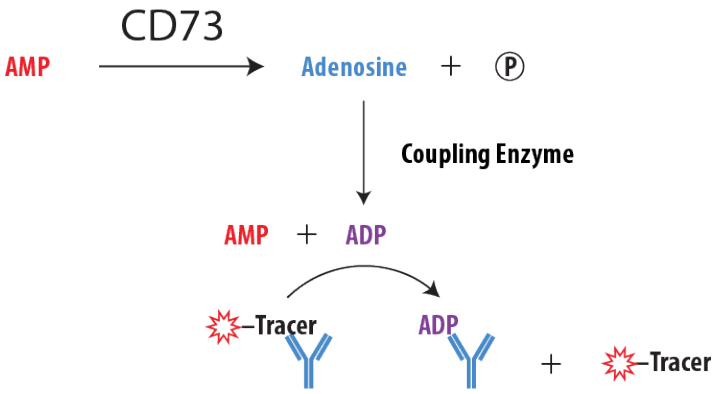
Enzyme Target: CD73

Detection Formats: FP

Discover Novel CD73 Inhibitors

The Transcreener CD73 Assay measures adenosine (ADO) produced by ecto-5′-nucleotidase (also known as 5′-nucleotidase, NT5E, Cluster of Differentiation 73 or CD73).

By measuring the production of ADO, researchers can effectively determine the activity of the enzyme. The assay provides an enabling method to screen compound libraries for CD73 modulators to help find new therapeutics for disease.



Applications

- Measure enzymatic activity of CD73
- Screen compound libraries for CD73 inhibitors
- Quantify inhibitor potency (IC_{50})
- Inhibitor selectivity profiling

Features

- Detection of unlabeled adenosine
- Easy-to-use, homogenous, one-step format
- Robust Assay Z′ > 0.7 under initial velocity conditions
- Far-red fluorescent readout minimize compound interference
- A safe, non-radioactive method

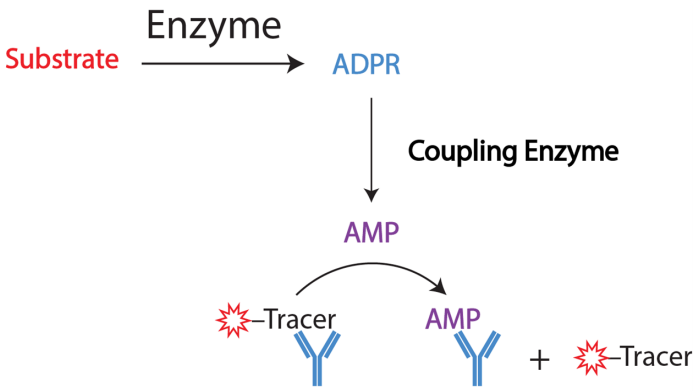
Transcreener® ADPR Assay

Enzyme Targets: CD38, PARG

Detection Formats: FP

Measure ADPR Activity with an HTS Assay

The Transcreener ADPR Assay is a biochemical HTS assay for measuring the production of ADP-ribose (ADPR) in enzyme reactions. The assay uses a coupling enzyme to convert ADPR into AMP, which is then detected using a far-red competitive fluorescence polarization assay.

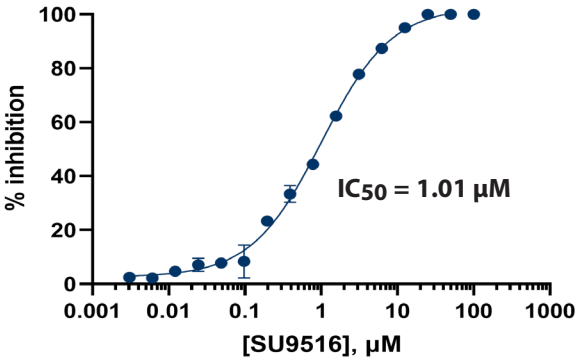


Target Focus: CD38

CD38 as a Therapeutic Target

CD38 is part of the extracellular CD38/CD203a/CD73/CD39 pathway to modulate adenosine production. CD38 is one of the first pathway steps, producing ADPR from NAD^+. Extracellular adenosine is a key player in the regulation of inflammation and immunogenicity.

CD38 Inhibitor Dose-Response



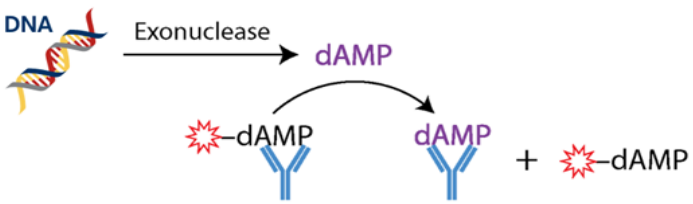
Transcreener® dAMP Assay

Enzyme Targets: Exonucleases, TREX1

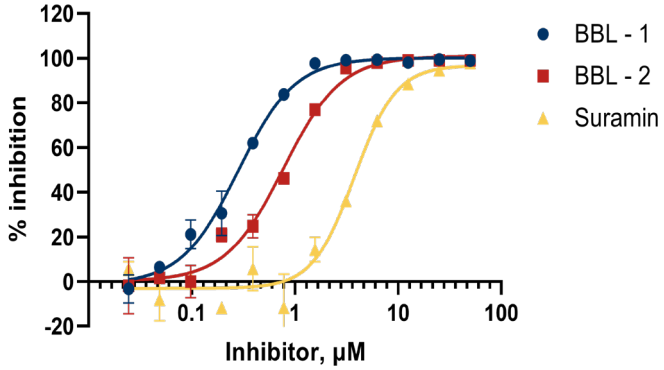
Detection Formats: FP

Discover Novel Exonuclease Inhibitors

The Transcreener dAMP Exonuclease Assay directly measures dAMP produced by exonuclease enzymes as they cleave polynucleotides. These dAMP measurements allow researchers to effectively determine the activity of the enzyme.



Obtain Dose-Response Data Fast



Applications

- Measure enzymatic activity of exonucleases
- Screen compound libraries for modulators
- Quantify inhibitor potency (IC_{50})
- Inhibitor selectivity profiling
- Measure drug-target residence time

Assay Systems (Sold Separately)

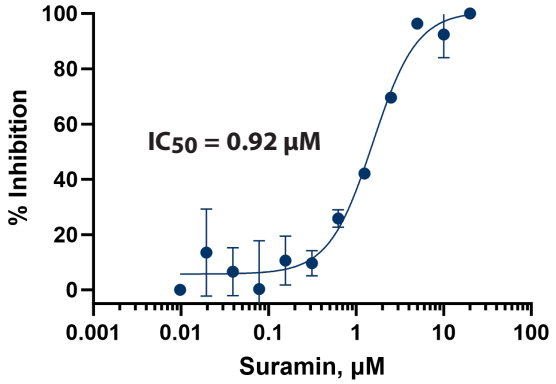
A Comprehensive Approach to Measure Enzymatic Activity and Screen & Profile Inhibitors

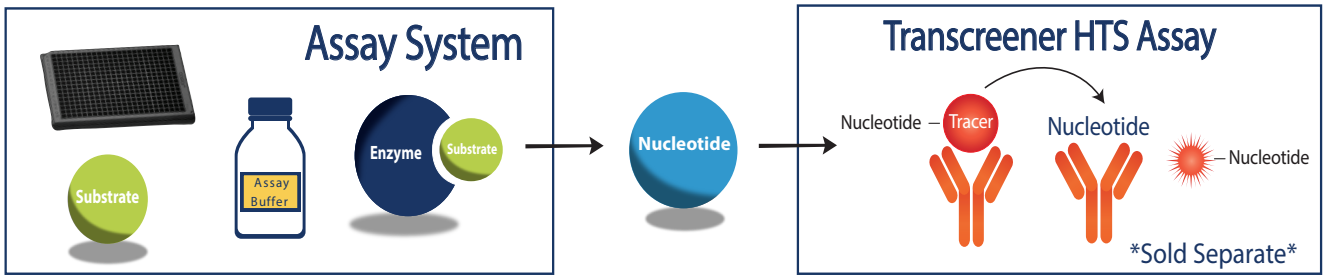
Assay Systems includes the enzyme, substrate, assay plates, and buffer components required to produce the enzyme reaction. They are sold separately from the Transcreener Assay Kits.

Current Available Assay Systems:

- **TREX1 -** TREX1 enzyme, DNA, plates, buffer
- **CD38 -** CD38 enzyme, NAD^+, plates, buffer
- **PARG -** PARG enzyme, polyADPR, plates, buffer
- **POLQ Helicase ATPase -** POLQ enzyme, ssDNA, plates, buffer
- **WRN Helicase ATPase -** WRN enzyme, WRN-H DNA, plates, buffer
- **RIG-I ATPase -** RIG-I enzyme, dsRNA, plates, buffer
- **MDA5 ATPase -** MDA5 enzyme, dsRNA, plates, buffer

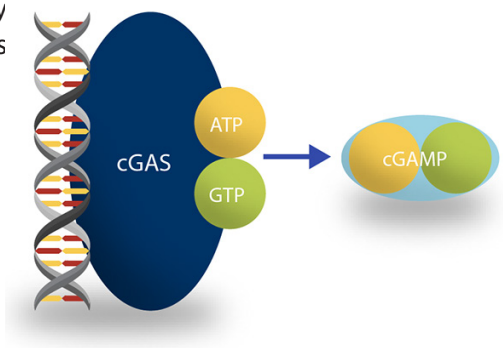
POLQ Inhibitor Dose Response





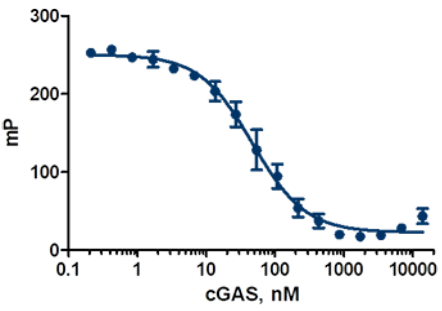
Active Enzymes (Sold Separately)

Having a purified active enzyme is critical to small molecule drug discovery efforts. Unlike enzymes from other commercial sources, each protein is verified for activity with a Transcreener assay to ensure quality data.



Current Available Enzymes:

- **cGAS, Human -** full-length, active, recombinant, human cGAS.
- **cGAS, Mouse -** full-length, active recombinant, mouse cGAS.
- **OAS1, Human -** full-length, active, recombinant OAS1.
- **DDX3, Human -** (AA 135-583), active, recombinant, DDX3.
- **TREX1, Human -** (AA 1-286), active, recombinant, TREX1.
- **CD38, Human** - (AA 1-269), active, recombinant, CD38.
- **PARG, Human** - (AA 1-976), full-length, active, recombinant, PARG.
- **POLQ Helicase, Human** - (AA 67-894), active, recombinant, POLQ helicase.
- **WRN Helicase, Human** - (AA 500-946), active, recombinant, WRN helicase.
- **RIG-I, Human** - (AA 1-925), full-length, active, recombinant, RIG-I.
- **MDA5, Human** - (AA 1-925), full-length, active, recombinant, MDA5.



Looking for an enzyme? Contact us to see if we can produce it or help you source one.

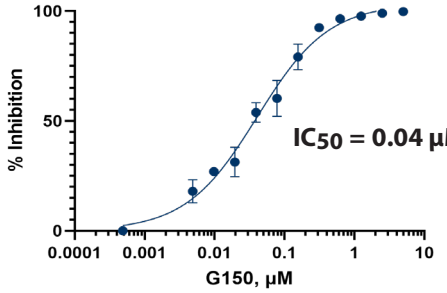
Targeting Innate Immunity

BellBrook leverages its assay technologies to build enabling products & services for DNA damage response & innate immunity targets. The innate immune response provides a first line defense against pathogens & tumor cells. The DNA damage response detects DNA lesions and mobilizes a variety of enzymes to repair the damage.

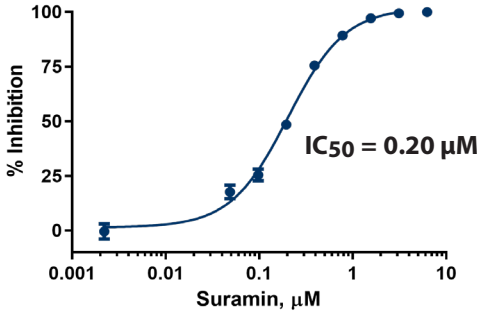
cGAS, OAS1

Transcreener **cGAS** Assay provides a robust, automatable method for screening, hit-to-lead and mechanistic studies focused on **cGAS** modulators to develop **cGAS** antagonists with nanomolar potency. The Transcreener 2-5A **OAS** Assay provides a simple HTS assay format to identify and characterize **OAS1** modulators, leveraging the trusted Transcreener AMP^2/GMP^2 Assay technology.

cGAS - Inhibitor Dose-Response



ENPP1 - Inhibitor Dose-Response



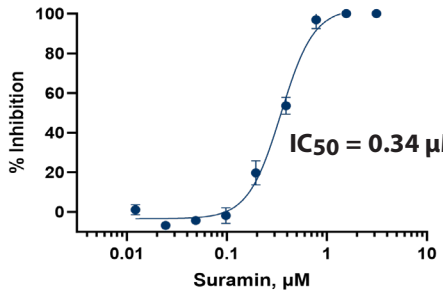
CD38, CD39, CD73, & ENPP1

Extrecellularly, adenosine (ADO) and cGAMP are critical regulators of the innate immune response with opposing effects. ADO is generated from ATP and ADP by **CD39** & **CD73**; and from NAD$^+$ by the combined action of **CD38**, CD203, and **CD73**. Extracellular cGAMP levels are controlled by the surface-exposed nucleotidase, **ENPP1**. Transcreener Assay kits are available for these 4 ectonucleotidases.

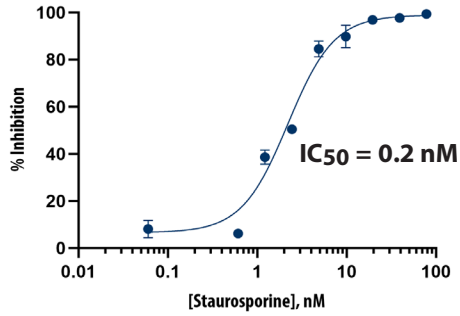
RIG-I, MDA5, & TREX1

RIG-I and **MDA5** are nucleic acid PRRs that detect dsRNA, triggering signaling cascades that produce type I interferons and pro-inflammatory cytokines. The Transcreener ADP^2 Assay measures **RIG-I** & **MDA5** activity. **TREX1** is an exonuclease that degrades dsDNA in the cytoplasm to prevent over-stimulation of cGAS. The Transcreener dAMP Assay measures **TREX1** activity. Assay Systems are also available for **RIG-1**, **MDA5**, & **TREX1**.

MDA5 - Inhibitor Dose-Response



AMPK - Inhibitor Dose-Response



AMPK, IKK, IRAKs, JAKs, TBK1 & More

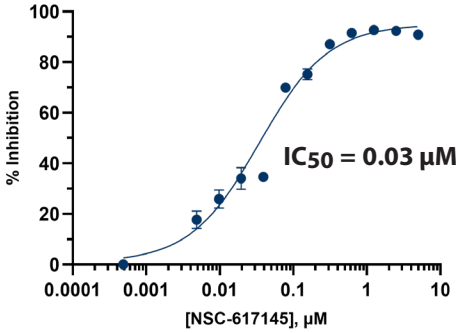
The Transcreener ADP^2 Kinase Assay has been used to capture millions of data points in HTS in both pharmaceutical and academic screenings. BellBrook has validated Transcreener with kinases in the cGAS/STING pathway, including **IKKβ** and **TBK1**. Both of these enzymes are also involved in signaling pathways for other types of pattern recognition receptors; eg, Toll-like receptors.

Targeting The DNA Damage Response

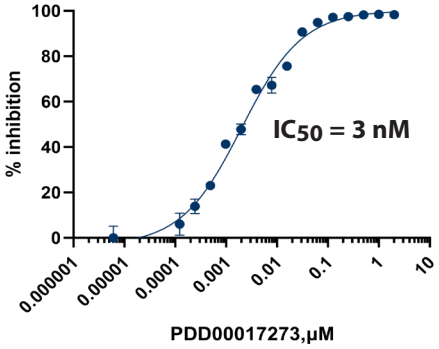
POLQ & WRN

POLQ and **WRN** proteins are multi-functional DNA-modifying enzymes involved in DSB repair. **POLQ** is synthetic lethal with BRCA-1 & ATM mutations, providing an alternative therapeutic strategy for PARPi-resistant tumors. **WRN** inhibitors are sought after therapeutics for targeting cancers that lack adequate mismatch repair machinery and enhanced mutation at microsatellite repeats (MSI-H). Screen for DDR modulators with the Transcreener ADP^2 Assay and associated **POLQ** & **WRN** Assay Systems.

WRN Helicase Inhibitor Dose-Response



PARG Inhibitor Dose-Response



PARG

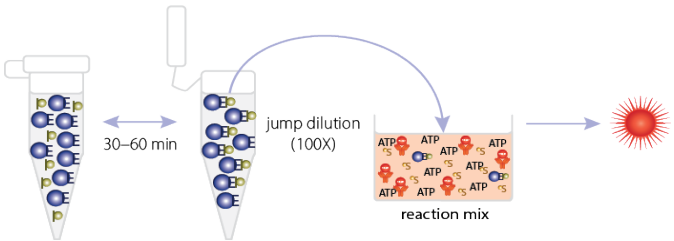
PARG hydrolyzes the ribose-ribose linkages in poly-ADP-ribose, releasing most ADPR monomers and some oligomers. Removal of poly-ADPR from PARP is critical for efficient DNA repair and replication. Additionally, ADPR produced by **PARG** can convert to ATP by NUDT5, which helps maintain nuclear ATP levels in DNA repair. The Transcreener ADPR Assay & **PARG** Assay System are innovative technologies to discover **PARG** antagonists.

Measuring Drug Residence Time

During drug development initiatives, analysis of drug-target residence times can improve efficacy, increase therapeutic window, and reduce risk of premature focus on compounds that likely have undesirable side effects. Transcreener biochemical assays provide a simple method to measure drug-target residence time in a HTS format.

Residence time (τ) is the time that a drug remains bound to its target before dissociating and is the reciprocal of dissociation rate (k_{off}). Residence time can be determined using a "jump dilution" method in which enzyme activity is monitored over time as an inhibitor dissociates.

Target Examples include: Kinases, ATPases, PDEs, and Glycosystransferases.

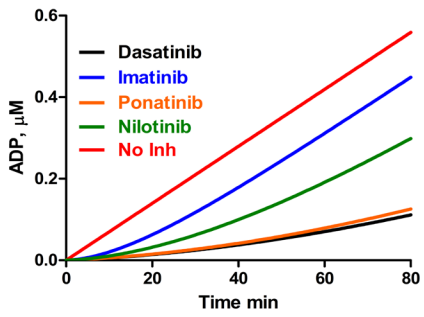


Step 1
- Incubate Enzyme with saturating concentration of Inhibitor

Step 2
- The [EI] complex is diluted into a solution containing saturating amounts of ATP and Substrate.
- Transcreener Detection reagents are added immediately

Step 3
- Following dilution, samples are read continuously to monitor change in Fluorescence over time.
- As the compound dissociates over time, the kinase activity is restored.

Step 4
- Determine koff by fitting enzyme progress curves to an integrated rate equation: [y = vs · t +(vi-vs/kobs) (1 - e-kobs · t/kobs)]



	Koff, min-1	Tau, min
Dasatinib	0.004011	249
Imatinib	0.05884	17
Ponatinib	0.004882	205
Nilotinib	0.0201	50

Universal, high throughput screening platforms for enzymes based on the detection of enzyme products

- **Universal assays cover thousands of target enzymes, including nearly any kinase, ATPase, GTPase, methyltransferase, or PDE**
- **Direct detection reduces potential for compound interference**
- **Far-red FP, FI, and TR-FRET readouts validated on major multi-mode readers**
- **Mix-&-read format with overnight deck & signal stability for easy automation**
- **Compatible with 96, 384, and 1536+ well formats for efficient HTS**

Biochemical Assay Kits

Assay Kit	Catalog #
Transcreener ADP^2 FP Assay	3010
Transcreener ADP^2 FI Assay	3013
Transcreener ADP^2 TR-FRET Assay	3011
Transcreener AMP^2/GMP^2 FP Assay	3015
Transcreener AMP^2/GMP^2 TR-FRET Assay	3020
Transcreener ADO FP Assay	3026
Transcreener cGAMP FP Assay	3024
Transcreener GDP FP Assay	3009
Transcreener GDP FI Assay	3014
Transcreener GDP TR-FRET Assay	3021
Transcreener UDP^2 FP Assay	3018
Transcreener UDP^2 TR-FRET Assay	3022
Transcreener EPIGEN SAH FP Assay	3017
Transcreener 2-5A FP Assay	3027
Transcreener dAMP Exonuclease Assay	3028
Transcreener ADPR FP Assay	3030
AptaFluor SAH TR-FRET Assay	3023

Assay Systems

Assay System	Catalog #
TREX1 Assay System	3029
CD38 Assay System	3031
WRN Helicase ATPase Assay System	3032
PARG Assay System	3033
POLQ Helicase ATPase Assay System	3034
RIG-I ATPase Assay System	3035
MDA5 ATPase Assay System	3036

Active Enzymes

Enzyme	Catalog #
Human cGAS	2227, 2228
Mouse cGAS	2239, 2300
OAS1	2249
DDX3	2251, 2252
TREX1	2260, 2262, 2265
CD38	2274, 2275
PARG	2283, 2285
POLQ Helicase	2287, 2289
WRN Helicase	2276, 2279
RIG-I	2291, 2294
MDA5	2298, 2299

Transcreener Assays are available in 1,000 and 10,000 data points based on 384-well plates.

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