

Optimize the Transcreener™ Assay for your Target Kinase

Maximize assay window and sensitivity by amending the ADP antibody concentration.

This guide is a supplement to the Transcreener™ KINASE Assay technical manual. Please read section 7.0 of the technical manual first.

The Transcreener HTS Assay platform is a highly flexible format and can detect kinase/ATPase activity over a wide range of starting ATP concentrations (1 μM -500 μM) in diverse reaction buffers. The Transcreener™ KINASE Assay Technical Manual and Certificate of Analysis offer suggestions for [ADP Antibody] using common kinase reaction conditions. These recommendations were based on the amount of antibody that provided the greatest mP shift at 10% ATP conversion in 50 mM HEPES, pH 7.5, 4 mM MgCl_2 , 2 mM EGTA, 1% DMSO, 0.01% Brij-35, and ATP (1 μM , 10 μM , 50 μM or 100 μM). However, the end-user may wish to validate the Transcreener™ KINASE assay using ideal reaction conditions for the target enzyme. This guide will help optimize the Transcreener™ KINASE Assay for diverse enzyme reaction conditions and [ATP] (1 μM -500 μM).

Assay Overview:

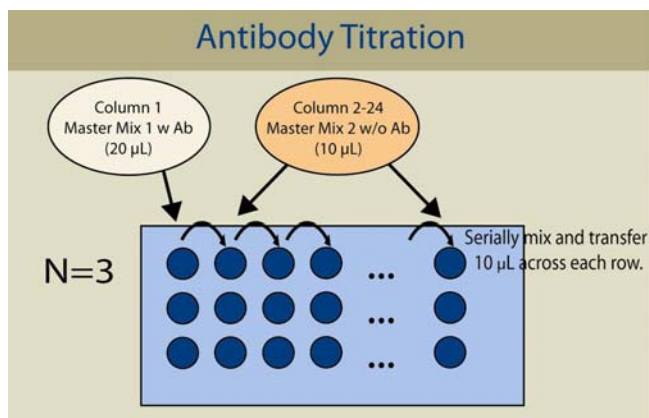
The Transcreener™ KINASE Assay is a two-part assay (a 10 μL enzyme reaction followed by the addition of 10 μL ADP Detection Mixture). The concentrations of the components in the 20 μL final volume are half, relative to either the enzyme reaction or the 1X ADP Detection Mixture.

Determine the ideal [ADP Antibody] for target enzyme reaction conditions:

Part 1: ADP Antibody Titration. Determine the amount of antibody that binds $\sim 85\%$ of the ADP far red tracer (EC_{85} value) in the target enzyme reaction buffer.

1. Prepare 2 Master Mixes in 1X enzyme reaction mix (include buffer, cofactors, substrate, ATP, etc.; exclude enzyme).
 - A. Master Mix 1** contains reaction components **with** ADP Antibody (1 mg/mL).
 - B. Master Mix 2** contains reaction components **without** ADP Antibody.

2. Dispense 20 μL of the **Master Mix 1** into the first column of a 384-well plate.
3. Dispense 10 μL of the **Master Mix 2** into columns 2-24 of the 384-well plate.
4. Remove 10 μL from column 1 and serially titrate the contents across the plate. Dispose of the 10 μL remaining.

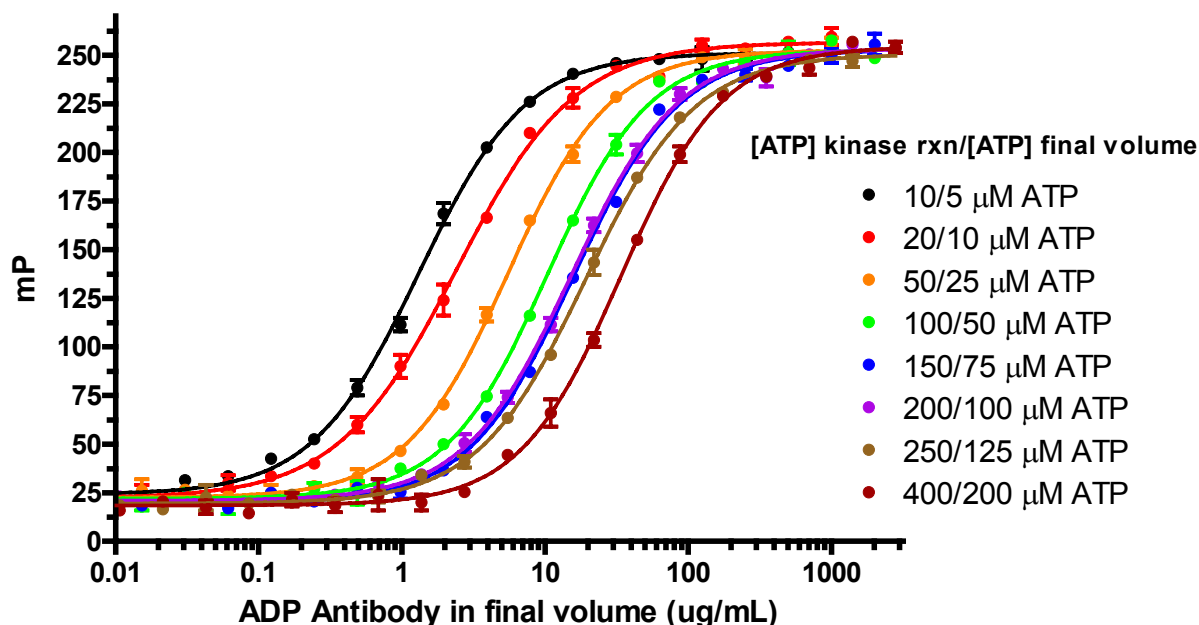


- Prepare a mixture of 1X Stop and Detect Buffer containing 1X ADP far red tracer(no antibody).
- Add 10 μ L 1X Stop and Detect Buffer containing 1X ADP far red tracer to the wells with titrated ADP Antibody. Mix the plate, equilibrate at room temperature (1hour), and measure fluorescence polarization.
- Plot mP vs [ADP Antibody]. Graph data using sigmoidal dose response curve fit. Use the equation below to determine the EC₈₅ value (where the EC₅₀ value and hillslope value are from the curve fit parameters).

$$EC_{85} = ((85 / (100 - 85))^{1/\text{hillslope}}) * EC_{50}$$

The [ADP Antibody] corresponding to the EC₈₅ value provides a near-optimum assay window at 10% ATP conversion. The end user may wish to proceed with their experiments using the [ADP Antibody] equal to the EC₈₅ value. However, **Part 2** of this guide explains how to maximize assay window and sensitivity.

ADP Antibody Titration with various [ATP]



Sample data: Antibody titration at various concentrations of ATP.

Part 2: ADP/ATP Standard Curve. To fine-tune the sensitivity of the Transcreener™ KINASE Assay, perform ADP/ATP Standard Curves (from 0% to 100% ATP conversion) with select ADP Antibody concentrations corresponding to the EC₇₅-EC₉₀. Any EC value can be determined using the equation in **Part 1** of this guide. In general, the assay will be more sensitive with less antibody.

1. Prepare 1X stock solutions for both ADP and ATP in your enzymatic reaction mixture (include buffer, cofactors, substrate, ATP, etc.; exclude enzyme).
2. Prepare an ADP/ATP Standard Curve specific for the [ATP] for the target reaction condition. A 100 μM ADP/ATP Standard Curve follows.

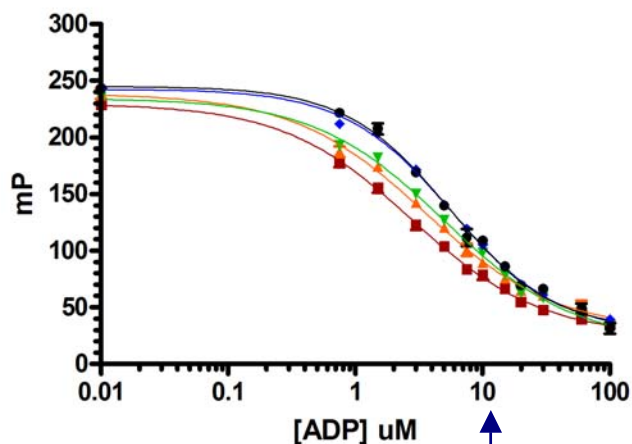
12 point Standard Curve Worksheet				
% ATP Conversion	Example 100 μM ADP/ATP μM	Sample volumes for 100 μL ADP/ATP	Calculate your concen. ADP/ATP	Calculate your volume ADP/ATP
0	0 μM /100 μM	0 μL /100 μL	μM / μM	μL / μL
2	2 μM /98 μM	2 μL /98 μL	μM / μM	μL / μL
4	4 μM /96 μM	4 μL /96 μL	μM / μM	μL / μL
6	6 μM /94 μM	6 μL /94 μL	μM / μM	μL / μL
8	8 μM /92 μM	8 μL /92 μL	μM / μM	μL / μL
10	10 μM /90 μM	10 μL /90 μL	μM / μM	μL / μL
15	15 μM /85 μM	15 μL /85 μL	μM / μM	μL / μL
20	20 μM /80 μM	20 μL /80 μL	μM / μM	μL / μL
30	30 μM /70 μM	30 μL /70 μL	μM / μM	μL / μL
40	40 μM /60 μM	40 μL /60 μL	μM / μM	μL / μL
60	60 μM /40 μM	60 μL /40 μL	μM / μM	μL / μL
100	100 μM /0 μM	100 μL /0 μL	μM / μM	μL / μL

3. Prepare 1X ADP Detection Mixture
Prepare 1X ADP Detection Mixtures (10 μL /well) with the predetermined ADP Antibody concentration from **Part 1** (EC₈₅). Also, prepare ADP/ATP Standard Curves with select ADP Antibody concentrations corresponding to the EC₇₅-EC₉₀. Performing several ADP/ATP Standard Curves with various [ADP Antibody] will allow you to determine the maximum assay window at various % ATP conversions
4. Measure fluorescence polarization: mix components, incubate for 1 hour at room temperature, and measure fluorescence polarization.

Part 3: Sample Data. The Transcreener™ KINASE Assay allows for great Z' values at low % ATP conversion. Therefore, the end-user must decide how far they wish their reaction to progress (% ATP conversion) and determine the amount of ADP Antibody that is ideal for their situation.

1. Calculate the change in fluorescence polarization (delta mP) for the ADP/ATP Standard Curve for each ADP Antibody concentration (EC₇₅-EC₉₀).
2. Select the [ADP Antibody] that generates the ADP/ATP Standard Curve with the greatest delta mP at the % ATP conversion of your choice. This [ADP Antibody] is ideal for your Transcreener™ assay.
3. Two sets of sample data follow (for a 100 μM ADP/ATP Standard Curve and a 1 μM ADP/ATP Standard Curve).

100 μM ATP Standard Curves

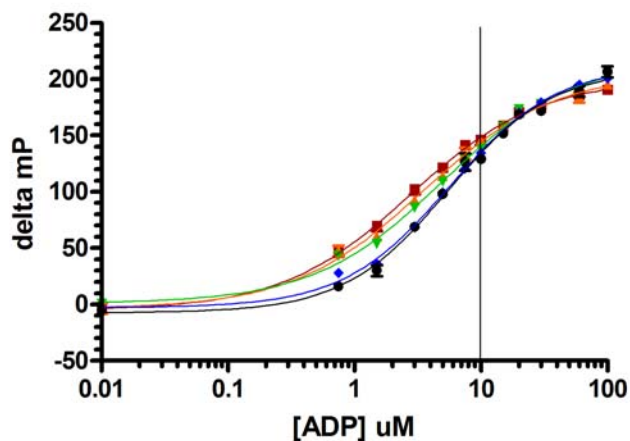


- 70 ug/mL Ab (EC80)
- ▲ 85 ug/mL Ab
- ▼ 100 ug/mL Ab (EC85)
- ◆ 120 ug/mL Ab
- 140 ug/mL Ab (EC90)

In the following example the starting $[\text{ATP}]$ is $100 \mu\text{M}$. Standard curves were generated using $100 \mu\text{M}$ ATP and varying $[\text{antibody}]$ chosen between $\text{IC}_{75} - 90$ and plotted against mP.

10% ATP conversion

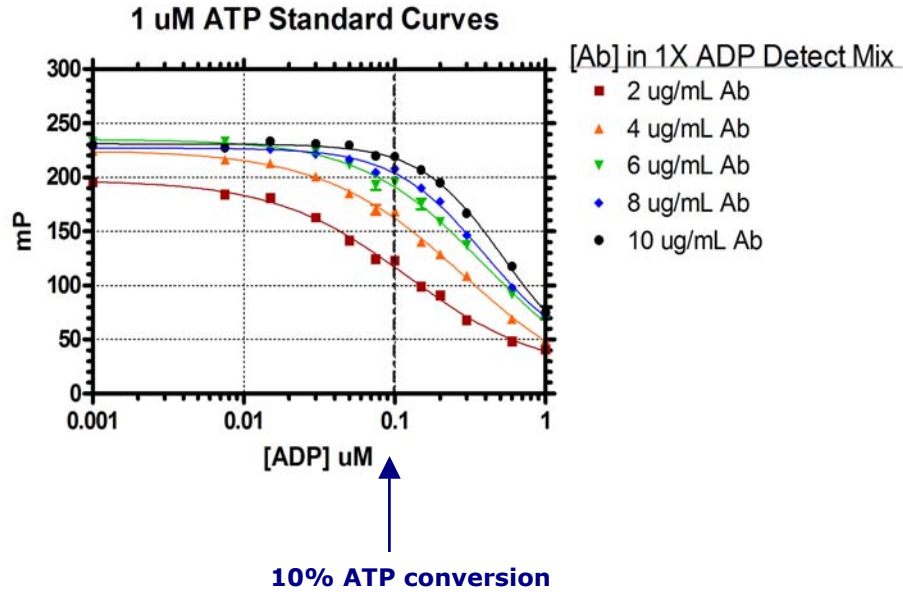
100 μM ATP Standard Curves



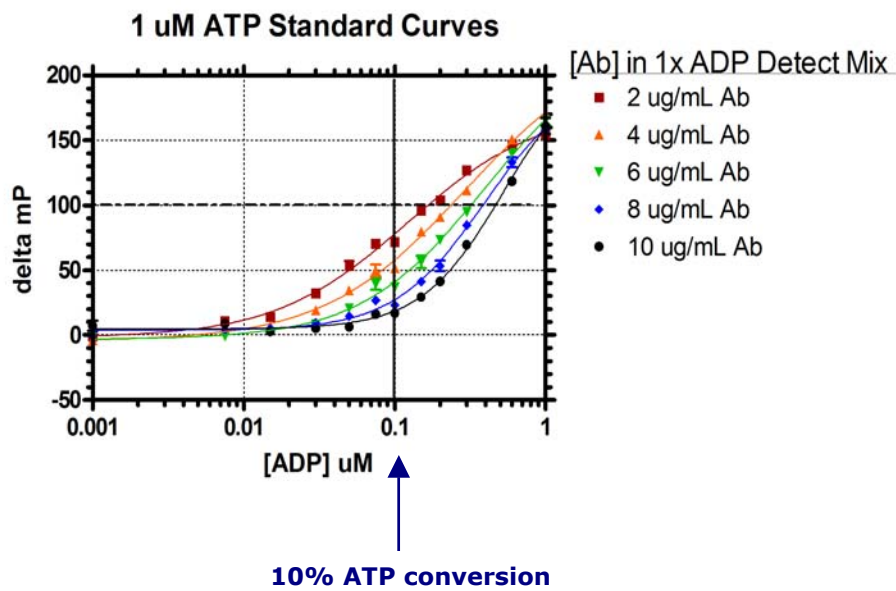
- 70 ug/mL Ab
- ▲ 85 ug/mL Ab
- ▼ 100 ug/mL Ab
- ◆ 120 ug/mL Ab
- 140 ug/mL Ab

Notice when plotted against delta mP, the shift remains high even at low ATP conversion rates.

10% ATP conversion



In the following example the starting [ATP] is 1 μM . Standard curves were generated using 1 μM ATP and varying [antibody] chosen between IC₇₅₋₉₀ and plotted against mP.



Notice when plotted against delta mP the lowest antibody concentration results in the greatest shift at the [ADP] representing 10% ATP conversion.