

## ab287855 – ADP sensor Universal Kinase Activity Assay Kit

For detection of kinase activity in purified/semi-purified/immuno-precipitated Kinases or crude cell/tissue extracts.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<https://www.abcam.com/ab287855>

### Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

### Materials Supplied

Item	Quantity	Storage Condition
Kinase Assay Buffer	25 mL	-20°C
ADP Sensor I	1 vial	-20°C
ADP Sensor II	1 vial	-20°C
Probe (in DMSO)	0.4 mL	-20°C
Ultra-pure ATP (ADP depleted)	1 vial	-20°C
DTT (1M)	100 µL	-20°C
ADP Standard	1 vial	-20°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (fluorescent plate reader)
- Desired Kinase substrate

### Reagent Preparation

- Read the entire protocol before performing the assay.
- Briefly spin the small vials prior to use.

**Kinase Assay Buffer:** Bring to room temperature (RT) before use. Store at 4°C or -20°C.

**ADP Sensor I & II:** Reconstitute each vial with 220 µL Kinase Assay Buffer separately. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze thaw. Keep on ice while in use. Stable for 2 months at -20°C.

**Probe:** Thaw the DMSO solution at room temperature before use. Aliquot and store at -20°C. Use within two months.

**Ultra-pure ATP:** Reconstitute with 220 µL dH<sub>2</sub>O to generate 5 mM ATP stock solution. Aliquot and store at -20°C. Keep on ice while in use.

**DTT:** Thaw the solution on ice before use. Aliquot and store at -20°C. Dilute to 1:500 to make a working solution of 2 mM. Always make a fresh working solution before use.

**ADP Standard:** Reconstitute with 0.5 mL dH<sub>2</sub>O to generate 2 mM ADP stock solution. Aliquot and store at -20°C. Keep on ice while in use.

## Assay Protocol

### Sample preparation:

1. Add kinase samples (1-100 ng) into desired wells in a 96-well plate.
2. Adjust the volume to 20 µL with the Kinase Assay Buffer.

### Δ Notes:

- a) Other kinases and small molecule metabolites (such as ADP, NADH etc.) may generate background. For accurate assays, we recommend using purified or partially purified sample without small molecule metabolites and other kinases. If crude cells/tissue lysate sample are used, either precipitate the sample with ammonium sulfate or spin the sample with 10 kDa filter to remove small molecule metabolites. Sample background may be subtracted by running a Background Control.
- b) For optimal performance, the temperature of the reaction, the amount of kinase enzyme and the desired substrate concentration may be optimized as needed.
- c) If other reaction buffer compositions are preferred, it is recommended to keep the MgCl<sub>2</sub> concentration within 1-20 mM.
- d) DTT may enhance some kinase activities, in such case, 1-2 µL of freshly diluted DTT may be added into the Kinase Sample preparation. When DTT is added, we recommend performing the kinase reaction at room temperature, not at 37°C

### Standard Curve Preparation:

1. Freshly dilute 5 µL of the 2 mM ADP stock solution with 495 µL dH<sub>2</sub>O to make 20 µM (20 pmol/µl) standard solution.
2. Add 0, 2, 4, 6, 8, 10 and 20 µL of 20 µM ADP Standard into a series of wells in a 96 well plate to generate 0, 40, 80, 120, 160, 200 and 400 pmol/well of ADP Standard.
3. Adjust final volume to 20 µL/well with Kinase Assay Buffer.

### Reaction Mix:

1. Just before running the assay, mix enough reagents (in the given order) for the number of assays (Kinase reactions and Standards) to be performed. For each well, prepare 30 µL Reaction Mix containing:

	Sample Reaction Mix	Background Control Mix <sup>a</sup>
Kinase Assay Buffer	10 µL	22 µL
Desired Kinase Substrate <sup>b</sup>	10 µL	-
Ultra-Pure ATP <sup>c</sup>	2 µL	-
ADP Sensor I	2 µL	2 µL
ADP Sensor II	2 µL	2 µL
Probe	4 µL	4 µL

2. Add 30 µL of the Reaction Mix to ADP standard and reaction wells, Background Control Mix to sample background control wells. Mix well.

### \*Δ Notes:

- a) For samples generating high background, prepare a Background Control well(s). The background readings can be subtracted from the sample readings.
- b) For the Standards, replace 10 µL Kinase substrate with 10 µL Kinase Assay Buffer.
- c) Use only the Ultra-pure ATP (ADP-depleted) provided. Other sources of ATP may generate high background.

## Measurement

Measure fluorescence (Ex/Em = 535/587 nm) either kinetically or in end point mode for 60-120 min. For kinetic reading, choose two time points (T1 & T2) in the linear range (RFU1 & RFU2). The ADP Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

**Δ Note:** *The incubation time will depend on the amount of kinase activity in the reaction; incubate longer if the kinase activity is low.*

## Calculation

Subtract the 0 Standard reading from all readings. Plot the ADP Standard Curve. Apply the ΔRFU (RFU2-RFU1) to the Standard Curve to get B pmol of ADP generated by Kinase during the reaction time (ΔT = T2 - T1). If sample background control reading is significant, subtract the ΔRFU value (calculated for the same T2 and T1 as the sample) of the background control from those of the test samples to determine the background-corrected change in fluorescence intensity for each well.

**Sample Kinase Activity =  $B/C \times \Delta T$  = pmol/min/ng = μmol/min/mg = U/mg**

Where:

**B** is the ADP amount from the Standard Curve (pmol)

**C** is amount of protein (ng)

**ΔT** is reaction time (min.)

**Unit Definition:** One unit of Kinase is the amount of kinase generates 1.0 μmol of ADP per min at pH 7.2 at room temperature or at 37°C.

## Technical Support

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