Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

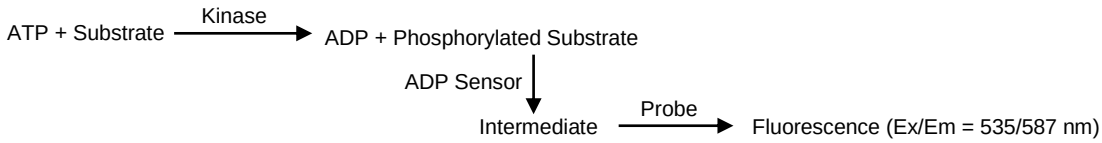
ADPsensor™ Universal Kinase Activity Assay Kit

10/15

(Catalog # K212-100; 100 assays; Store at -20°C)

I. Introduction:

Protein Kinases belong to a large family of phosphotransferases that mediate a wide spectrum of metabolic events via signal transduction pathways. Protein Kinases catalyze transfer of a phosphate group from a phosphate donor to a substrate protein. Kinases modulate the function of numerous proteins and are well-known therapeutic targets in many diseases like cancer, inflammation and diabetes etc. BioVision's ADPsensor™ Universal Kinase Assay Kit is based on the fluorescence detection of ADP generated by Kinase activity. In this assay, ADP formed during the kinase reaction is converted to an intermediate by the ADP Sensor Mix, which then reacts with a probe to generate a strong and stable fluorescence signal at Ex/Em = 535/587 nm. ATP in the kinase reactions doesn't interfere with the assay; therefore our kinase assay can be read kinetically (or at endpoint). The kit provides a universal tool to measure kinase activity in presence of substrates of your interest, or to screen/characterize kinase inhibitors. The assay is simple, non-radioactive, highly sensitive (<0.05 µM ADP) with broad ATP tolerance (up to 1 mM). The kit can detect as low as 1 ng of active Kinase.



II. Application:

- Measurement of Kinase activity
- Characterizing kinase substrates
- Screening/study/characterizing kinase inhibitors

III. Sample Type:

- Purified/semi-purified/immuno-precipitated Kinases
- Crude cell/tissue extract

IV. Kit Contents:

Components	K212-100	Cap Code	Part Number
Kinase Assay Buffer	25 ml	WM	K212-100-1
ADP Sensor I	1 Vial	Purple	K212-100-2
ADP Sensor II	1 Vial	Red	K212-100-3
Probe (in DMSO)	0.4 ml	Blue	K212-100-4
Ultra-pure ATP (ADP depleted)	1 Vial	Orange	K212-100-5
DTT (1M)	100 µl	Green	K212-100-6
ADP Standard	1 Vial	Yellow	K212-100-7

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Once opened, store the kit components as per the respective temperatures mentioned below. Read the entire protocol before performing the assay.

- **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_2O 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_2O to generate 2 mM ADP stock solution. Aliquot and store at -20°C. Keep on ice while in use.

VII. Kinase Assay Protocol:

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

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 (Cat #1997-25) to remove small molecule metabolites. Sample background may be subtracted by running a Background Control in step 3.

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 $MgCl_2$ 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.

2. Standard Curve Preparation: Freshly dilute 5 µl of the 2 mM ADP stock solution with 495 µl dH2O to make 20 µM (20 pmol/µl) standard solution. 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. Adjust final volume to 20 µl/well with Kinase Assay Buffer.

3. Reaction Mix: 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[a]
Kinase Assay Buffer	10 µl	22 µl
Desired Kinase substrate[b]	10 µl	--
Ultra-Pure ATP[c]	2 µl	--
ADP Sensor I	2 µl	2 µl
ADP Sensor II	2 µl	2 µl
Probe	4 µl	4 µl

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

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.

4. 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).

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

5. 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.

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

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.

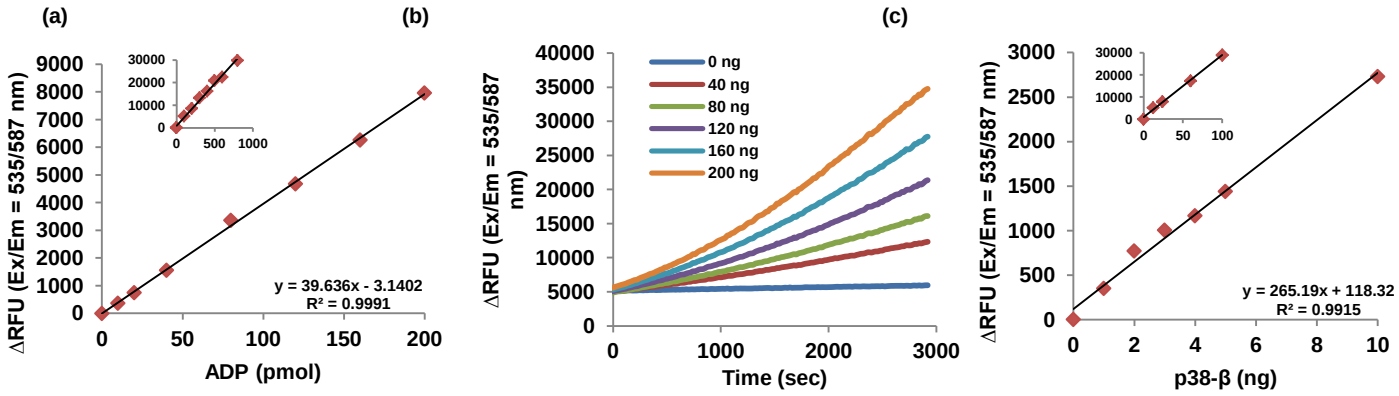


Figure: (a) ADP Standard Curve (0-200 pmol and 0-1000 pmol) (b) Measurement of Kinase activity of 40-200 ng of Recombinant Her2 (Cat. # 8011), (c) Measurement of Kinase activity of 1-100 ng of p38-β (Cat # 7763-5). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

ADP Colorimetric/Fluorometric Assay Kit (K355)
ADP/ATP Ratio Bioluminescence Assay Kit (K255)
PicoProbe™ ADP Assay Kit (Fluorometric) (K211)
HER2, Active, Human Recombinant (8011)

FOR RESEARCH USE ONLY! Not to be used on humans