

ADP-Lite Kinase Assay

DD1230



Instruction for Use
Version 26.2

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For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

ADP-Lite Kinase Assay is a universal kinase activity assay kit designed for measuring the activity of any ADP-generating enzyme (e.g., kinases and ATPases). To accurately determine target kinase activity, the assay quantifies the ADP produced during a kinase reaction, which is positively correlated with the kinase activity. This simple, rapid, and homogeneous assay is based on a highly sensitive in vitro bioluminescence technology that requires no excitation light or radioactive materials. It is compatible with a wide range of kinase substrates, including lipids, peptides, proteins, and carbohydrates.

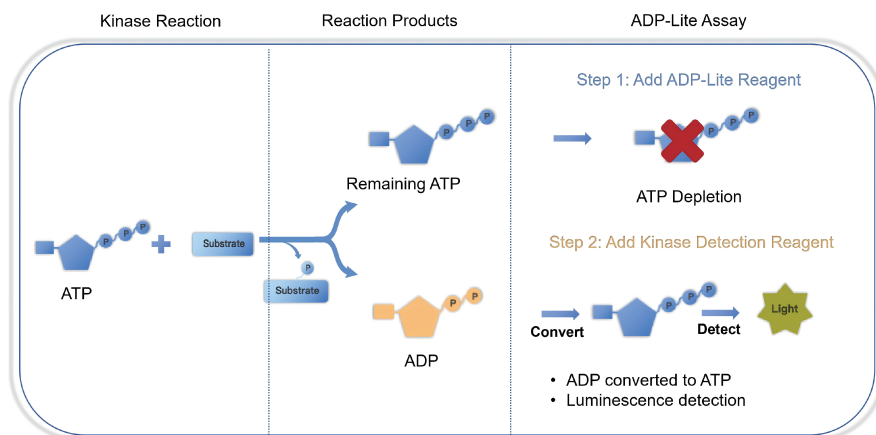


Figure 1. Assay Principle

The kit contains two core components: ADP-Lite Reagent (for ATP depletion) and Kinase Detection Reagent (for ADP detection). Kinase Detection Reagent is prepared by mixing Kinase Detection Buffer and Kinase Detection Substrate (Lyophilized). After the kinase reaction, ADP-Lite Reagent is added to deplete any remaining ATP. Kinase Detection Reagent is then added to terminate ATP depletion and convert the ADP generated by the kinase reaction to ATP. Finally, the luminescence signal produced from luciferase-luciferin and ATP is measured using a microplate reader to quantify kinase activity (Figure 1). The kit is suitable for kinase reactions with ATP concentrations ≤ 1 mM. Luminescence signals are stable for up to 3 hours (coefficient of variation (CV) < 20%), ensuring reliable and reproducible results.

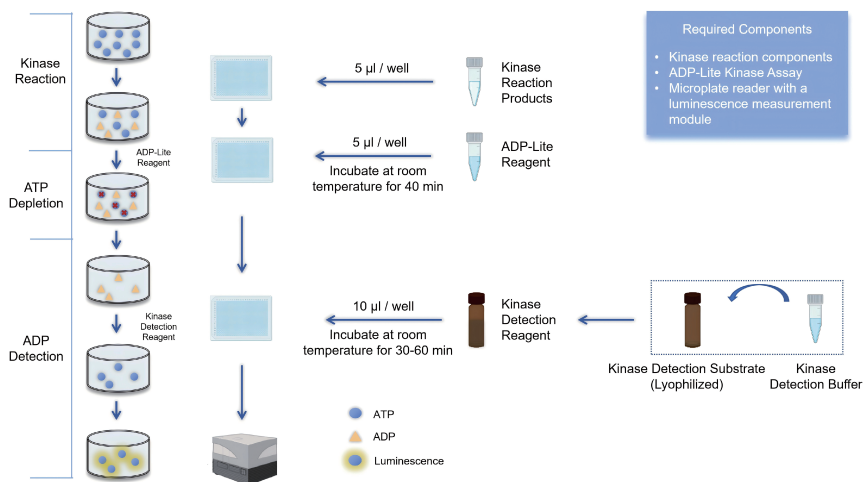


Figure 2. Assay Workflow

02/Components

Components	DD1230-01 (400 assays)	DD1230-02 (1,000 assays)	DD1230-03 (10,000 assays)
ADP-Lite Reagent	2 ml	5 ml	50 ml
Kinase Detection Buffer	4 ml	10 ml	100 ml
Kinase Detection Substrate (Lyophilized)	1 vial	1 vial	1 vial
Ultra Pure ATP, 10mM	500 µl	500 µl	5 ml
ADP, 10mM	500 µl	500 µl	5 ml

For 384-well plates (5 µl:5 µl:10 µl), DD1230-01 is sufficient for 400 assays; DD1230-02 is sufficient for 1,000 assays; DD1230-03 is sufficient for 10,000 assays.

For 96-well plates (25 µl:25 µl:50 µl), DD1230-01 is sufficient for 80 assays; DD1230-02 is sufficient for 200 assays; DD1230-03 is sufficient for 2,000 assays.

03/Storage

Store at -30 ~ -15°C and ship at ≤ 0°C; protect from direct light.

04/Applications

Determination of kinase activity; profiling of kinase activators and inhibitors, including mechanism of action (MOA) studies to distinguish between ATP-competitive and non-competitive inhibitors; identification of compounds that selectively target kinases.

05/Self-prepared Materials

Single- or multi-channel pipettes;

Solid white or solid black 96-well or 384-well plates;

Kinase reaction components (kinase, substrate, kinase reaction buffer);

Microplate reader with a luminescence measurement module.

06/Notes

1. After removing from -20°C storage, allow reagents to equilibrate to room temperature before use. If a precipitate is observed, incubate at 25°C and vortex to dissolve, or centrifuge briefly and use the supernatant.
2. It is not necessary to add a separate stop reagent to terminate the kinase reaction. If a stop reagent is required to terminate the reaction early for specific experiments, avoid using magnesium-chelating agents such as EDTA.
3. For kinase activity assays (including inhibitor screening), maintain a volume ratio of 1:1:2 for kinase reaction components to ADP-Lite Reagent to Kinase Detection Reagent.
4. To prevent well-to-well signal crosstalk, use of solid white or solid black 96-well or 384-well plates is recommended.
5. The kit needs to be used together with a microplate reader equipped with a luminescence measurement module. Measured signals may vary between different instrument models due to differences in parameter settings and sensitivity across manufacturers.
6. The luminescence spectrum of luciferase reaction is wide. A full wavelength scan can be selected to achieve higher sensitivity in detecting luminescence signals. If the mode is unavailable, set the detection wavelength to 560 nm.

07/Experiment Process

07-1/Reagent Preparation

1. Thawing: Remove ADP-Lite Kinase Assay reagents from -20°C storage. Thaw completely and equilibrate to room temperature.
2. Preparation of Kinase Detection Reagent: Add Kinase Detection Buffer to Kinase Detection Substrate (Lyophilized). Mix thoroughly until fully dissolved to obtain the Kinase Detection Reagent.

▲ After removing the required volume for your experiment, aliquot any unused reagents and return immediately to -20°C. ADP-Lite Reagent and Kinase Detection Reagent remain stable with no significant loss of signal after 10 freeze-thaw cycles or storage at room temperature (25°C) for 24 hours.

- If needed, generate an ATP-to-ADP conversion standard curve. Prepare 1 ml of 1 mM ATP and 500 μ l of 1 mM ADP in 1 $\times$ kinase reaction buffer. Combine the solutions according to the table below to generate a 1 mM standard series.

Well Number	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
1 mM ADP (μ l)	100	80	60	40	20	10	5	4	3	2	1	0
1 mM ATP (μ l)	0	20	40	60	80	90	95	96	97	98	99	100

To generate standard curves at other concentrations, prepare ATP and ADP at the desired concentrations or perform serial dilutions from the 1 mM series.

07-2/Protocol

Example using a 384-well plate (Figure 2):

- Kinase Reaction: This kit does not provide the kinase, substrate, or 1 $\times$ kinase reaction buffer. The Ultra Pure ATP supplied in the kit can be used for the reaction. Note: If the kinase reaction is not performed at room temperature, equilibrate the plate to room temperature before adding ADP-Lite Reagent.
- Terminate Kinase Reaction and Deplete ATP: Add 5 μ l of ADP-Lite Reagent to each well and mix gently. ADP-Lite Reagent is effective in terminating the kinase reaction, and there is no need to add a separate inhibitor. Incubate at room temperature for 40 min to ensure complete ATP depletion.
- Convert ADP to ATP: Add 10 μ l of Kinase Detection Reagent to each well and mix gently. This converts ADP to ATP and uses a luciferase-luciferin reaction to detect ATP.
- Incubation & Signal Development: Incubate at room temperature for 30 - 60 min. Adjust the incubation time based on the ATP concentration used in the kinase reaction:

ATP Concentration	ATP < 100 μ M	100 μ M $\leq$ ATP < 500 μ M	ATP $\geq$ 500 μ M
Incubation Time	30 min	40 min	60 min

- Read the luminescence signal from each well using a microplate reader (luminescence detection mode).

IC₅₀ Determination for Kinase Inhibitors in a 384-Well Plate

- Add 2 μ l of 1 $\times$ kinase reaction buffer to wells in columns 2 - 12.
- Add 4 μ l of the inhibitor (diluted to the highest concentration desired in 1 $\times$ kinase reaction buffer) to wells in column 1.
- Transfer 2 μ l from the wells in column 1 to column 2 and mix well. Continue to make 2-fold serial dilutions across the plate.
- Add 2 μ l of 2.5 $\times$ kinase to wells in columns 1 - 12 and mix well.
- Add 1 μ l of the mixture of 5 $\times$ substrate and 5 $\times$ ATP to wells in columns 1 - 12 and mix well.
- Incubate at the optimal temperature for the required amount of time.
- Follow the assay procedure described above.



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