Version 1 Last updated 25 May 2018

ab228557 Adenylate Kinase Cytotoxicity Assay Kit

For the measurement of Adenylate Kinase released from damaged cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Adenylate Kinase Cytotoxicity Assay Kit (ab228557) is based on the measurement of AK in a simple one-step procedure involving two chemical reactions. The first reaction converts ADP to ATP by adenylate kinase released from damaged cells. The second reaction utilizes luciferase to catalyze the formation of light from ATP and luciferin; the light is then measured using a luminometer or beta counter. The assay is highly sensitive and can be fully automated for high throughput applications.

Cell death or cytotoxicity is classically evaluated by the quantification of plasma membrane damage. Adenylate kinase (AK) is a ubiquitous protein present in all eukaryotic and prokaryotic cells and rapidly released into culture medium upon damage to the plasma membrane.

Treat cells by desired method. Use control culture without treatment.

Add samples and controls to appropriate wells.

Add AK Detection Reagent Working Solution to each well. Incubate for 5 min.

Read in Microplate Luminometer within 30 min

2. Materials Supplied and Storage

Store kit at -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
AK Detection Reagent Lyophilized	5 vials	-20°C	-20°C
AK Assay Buffer	50 mL	-20°C	-20°C

3. Materials Required, Not Supplied

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

- Microplate Luminometer

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Read entire protocol before performing the assay.

5.1 AK Detection Reagent Stock Solution

Reconstitute a vial of the AK Detection Reagent with 1.1 mL AK Assay Buffer. Mix gently. Allow the mixture to equilibrate for 15 min at room temperature before use. Stock solution can be stored for 24 hours at 4°C.

5.2 AK Detection Reagent Working Solution

Dilute AK Detection Reagent Stock Solution 10-fold, depending upon the number of samples and controls to be measured. Each well requires 100 µL of Working Solution. Use diluted reagent within the same day. Once reconstituted, the AK Detection Reagent must not be refrozen.

6. Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

6.1 Treat cells by desired method. Concurrently incubate a control culture without treatment.

6.2 Transfer 100 µL of the culture medium into each well if using a 96 well plate. If using a 384-well plate, we recommend adding 20 µL of the culture medium to each well.

6.3 Add 100 µL of the AK Detection Reagent Working Solution to each well. Incubate for 5 minutes. If using a 384-well plate, we recommend adding 30 µL of the AK Detection Reagent Working Solution to each well.

6.4 Read in a Microplate Luminometer. Samples should be read within 30 minutes following the addition of the AK Detection Reagent Working Solution. The reaction time should be kept consistent for all samples. The reaction can also be followed kinetically.

Δ Note If using a microplate luminometer equipped with reagent dispensers, the Dispenser should be primed with the AK Detection Reagent Working Solution and set to dispense 100 µL (96 well plate) or 30 µL (for 384 well plate). It is recommended that a delay time of at least 5 minutes prior to measurement (but no more than 30 minutes) be incorporated after injection of the AK Detection Reagent Working Solution. 1 second integrated reading is recommended.

7. Notes

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