Product Information

AK Bioluminescence Cytotoxicity Assay Kit

I. Kit Contents:

Components	K2026-500 500 assays	Cap Color	Part Number
AK Detection Reagent (lyophilized)	5 vial	Green	K2026-C-1
AK Assay Buffer	50 ml	NM	K2026-C-2

II. Introduction:

Cells treatment with the cytotoxic compound may undergo necrosis, in which they lose membrane integrity. Hence cytotoxicity can be detected by the quantification of plasma membrane damage. Adenylate kinase (AK) is a phosphotransferase enzyme that ubiquitously present in all prokaryotic and eukaryotic cells. When damage of the plasma membrane, AK is rapidly released into the cell culture medium.

The AK Bioluminescence Cytotoxicity Assay Kit provides a simple and fast way for the quantification of cytotoxicity based on the measurement of AK involving two chemical reactions. First, AK that is released from the damaged cells converts ADP to ATP. Second, luciferase catalyzes the formation of light from luciferin and ATP, and the light can be detected using a beta counter or luminometer. The assay is highly sensitive and can be fully automatic for high throughput.

III. Preparation of Reagents and General Considerations:

AK Stock Reagent: 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℃. One vial is sufficient for 100 wells. AK Detection reagent in lyophilized form can be stored at –20℃ for up to 2 months.

AK Detection Reagent Working Solution: Dilute AK Stock Reagent 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 Detection Reagent must not be refrozen. Ensure that all reagents are at room temperature before use. The optimal temperature is 22℃.

IV. Assay Protocol:

1. Treat cells by desired method. Concurrently incubate a control culture without treatment.
2. Transfer 100 µl of the culture medium into a 96 well plate.
3. Add 100 µl of the AK Reagent Working Solution to each well. Incubate for 5 minutes.

Note: If using a 384-well plate, we recommend adding 20 µl of the culture medium and 30 µl of the AK Reagent Working Solution.

4. Read in a Microplate Luminometer.

Note: Samples should be read within 30 minutes following the addition of the AK Reagent Working Solution. The reaction time should be kept consistent for all samples. The reaction can also be followed kinetically.

V. Microplate Luminometers with Injectors:

If using a microplate luminometer equipped with reagent dispensers, the Dispenser should be primed with the AK Reagent Working Solution and set to dispense 100 µl (96well 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 Reagent Working Solution. 1 second integrated reading is recommended.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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