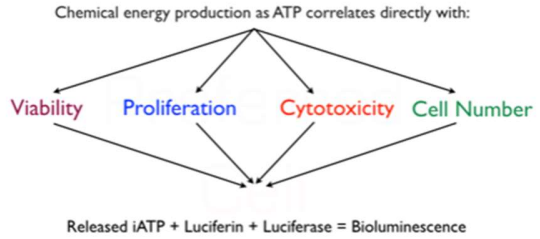
GenieGlow Cytotoxicity Assay Kit (luminescence) (BN00579)

(Catalog BN00579; 500 assays; Store at –20° C)

I. Introduction:

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. The **GenieGlow Cytotoxicity Assay Kit** 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.



II. Kit Contents:

Component	BN00579 500 assays	Cap Color
AK Detection Reagent (lyophilized)	5 vials	Green
AK Assay Buffer	50 ml	NM

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°C. One vial is sufficient for 100 wells. AK Detection reagent in lyophilized form can be stored at –20°C 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 100µl 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°C.

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.