Phosphate Assay Kit (Fluorometric)

(**Catalog # BN00669; 100 assays; Store at -20°C**)

I. Introduction:

Inorganic phosphate (Pi) is one of the most important ions in biological systems. It functions in a variety of roles. One of its most important roles is as a molecular switch, turning enzyme activity on and off through the mediation of the various protein kinases and phosphatases in biological systems. A highly sensitive assay is desired to monitor Pi in a variety of samples. The **newly designed and enhanced** Phosphate Assay Kit provides a sensitive, easy and quick means of assessing Pi over a wide range of concentrations. In this assay, inorganic phosphate reacts with sucrose to produce glucose-1-phosphate in the presence of a proprietary enzyme. The glucose-1-phosphate is specifically oxidized to generate a product that reacts with the assay to generate fluorescence (Ex/Em = 535/587 nm). The kit can be used to detect Pi produced through reactions involving ATPases, GTPases, 5'-nucleotidase, protein phosphatases, acid and alkaline phosphatases, phosphorylase, etc. from a variety of samples. Unlike other commercially available assays, this assay is not affected by the presence of glucose in samples. This kit can determine phosphate concentrations between 2 µM and 10 µM, with a lower detection limit of approximately 100 pmol.

II. Kit Contents:

Components	BN00669	Cap Code	Part Number
Phosphate Assay Buffer	25 ml	WM	BN00669-1
GenieProbe (in DMSO)	200 µl	Blue	BN00669-2
Converter	1 vial	Brown	BN00669-3
Developer	1 vial	Green	BN00669-4
Phosphate Substrate	220 µl	Purple	BN00669-5
Phosphate Standard (10 mM)	50 µl	Yellow	BN00669-6

III. Storage and Handling:

Store kit at –20ºC, protected from light. Allow the reagents to warm to room temperature and briefly centrifuge the vials prior to opening. Read the entire protocol before the assay. A 96-well white plate is recommended for this assay.

IV. Reagent Preparation:

GenieProbe (in DMSO): Store at –20ºC, protected from light and moisture. Use within two months.

Converter, Developer: Resuspend in 220 µl Assay Buffer, respectively. Aliquot and store at –70ºC. Avoid repeated freeze thaw. Use within two months.

V. Phosphate Assay Protocol:

Note: Phosphate contamination in samples must be carefully avoided. Laboratory detergents can contain large amount of phosphate, therefore glassware must be thoroughly rinsed with distilled water before use.

1. Standard Curve Preparations:

Dilute the Phosphate Standard to 100 µM by adding 10 µl of the Phosphate Standard to 990 µl of Assay Buffer. Mix well. Add 0, 2, 4, 6, 8 and 10 µl of 100 µM standard into a series of wells. Adjust the volume to 50 µl/well with Assay Buffer to generate 0, 200, 400, 600, 800, 1000 pmol/well of the Phosphate Standard.

2. **Sample Preparation:** Add 1 – 50 µl test samples in a 96-well plate; if using serum sample, serum (0.5 -2 µl/well) can be directly diluted in the Assay Buffer. Bring the volume to a total of 50 µl/well with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the linear range of the standard curve.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed: For each well, prepare a total of 50 µl Reaction Mix containing:

Assay Buffer	43 µl
GenieProbe	1 µl
Phosphate Substrate	2 µl
Converter*	2 µl
Developer	2 µl

***Note: You may do a control (optional) by omitting the Converter in the reaction, which will read the no-converter background. If the reading is higher than the 0 phosphate control, then the background should also be subtracted from Pi readings.**

4. Add 50 µl of the Reaction Mix to each well containing the Phosphate Standard, test samples and controls. Mix well.
5. Incubate the reaction for 1 hour at room temperature, protected from light.
6. Measure the fluorescence at Ex/Em = 535/587 nm in a micro plate reader.

7. Correct the background by subtracting the value derived from the 0 phosphate control from all readings. Plot the Pi Standard Curve. Apply the sample readings to the standard curve.

Pi Concentration = A/V pmol/µl or µM

Where: A is the Pi amount in the reaction from standard curve (in pmol),
V is sample volume added into the reaction well (in µl).

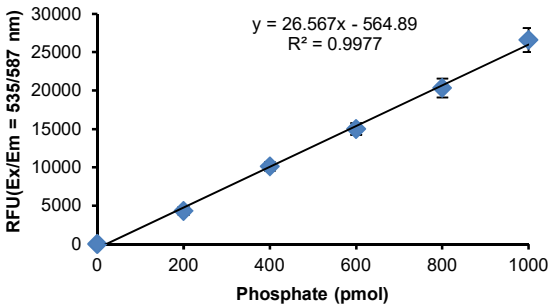

Figure: Phosphate standard curve made according to the protocol (n=3, error bars represent standard deviation).

FOR RESEARCH USE ONLY! Not to be used on humans.