Version 22-04/24

User's Manual

Glycerin detection kit

REF **DEIA-NS2310-13**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit can determine the glycerin content in biological product samples such as injections and vaccines.

Minimum detection limit: 0.01 mM

Effective linear range: 0.2 mM~2.0 mM

Results are accurate and repeatable

The kit is suitable for biomedical, biological products, and food laboratory testing.

Principles of Testing

Glycerin and NAD+ can be reduced to NADH under the action of glycerin dehydrogenase. The amount of NADH is directly related to the concentration of glycerin; this product calculates the amount of glycerin in the sample by detecting NADH at 340 nm.

Reagents And Materials Provided

Assay Buffer 1, 1.5 mL × 5 tubes, ≤-18°C

Assay Buffer 2, 100 µL × 2 tubes, ≤-18°C

Enzyme Mix, 0.5 mL × 2 tubes, ≤-18°C

100 mM glycerin standard, 200 µL × 1 tube, ≤-18°C

Note: The kit is stored at -18°C and below before unpacking. After unpacking and use, the product Assay Buffer 1 and 100 mM glycerin standard should be stored at 2-8°C. The product Assay Buffer and Enzyme Mix should be stored at -18°C or below, and cannot be frozen and thawed again for use after freezing and thawing twice.

Materials Required But Not Supplied

1. Applicable models (including microplate readers with a wavelength of 340nm, such as MD SpectraMax M2/i3, Thermo Varioskan Flash, BioTek SynergyMx/2)
2. ddH_2O
3. Centrifuge
4. Vortex shaker
5. Constant temperature incubator
6. 1000 µL, 100 µL, 10 µL pipettes

Storage

-18°C

Validity period: 6 months under specified storage conditions

Specimen Collection And Preparation

Direct measurement of clear liquid biological product samples such as injections and vaccines. If the initial measurement result exceeds the linear range of this kit, it needs to be diluted with ddH_2O and measured again. If you need a higher concentration standard for verification of dilution accuracy and spike recovery, you can purchase the national glycerin standard.

Preparation of high-concentration glycerin reference products

1. Place the 15 mL volumetric flask on the weighing balance and clear it to zero;
2. Slowly add the national glycerin standard and weigh 8.2881 g of glycerol;
3. Add double-distilled water to adjust the volume to 15 mL, shake and mix until the final concentration is 6 M, and then transfer it to a 15 mL centrifuge tube for storage.

Reagent Preparation

1. Standard dilution:

This kit contains a glycerin standard with a concentration of 100 mM. The dilution scheme is as follows:

Use ddH_2O to dilute 100 mM glycerin standard to 2.0 mM, 1.6 mM, 1.2 mM, 0.8 mM, 0.4 mM, 0.2 mM, 0.1 mM, and set a 0 mM concentration control reaction tube. See table for dilution scheme.

Tubes	Dilution volume	Concentration
ST1	20 μL 100 mM glycerin standard+ 980 μL ddH_2O	2.0 mM
ST2	800 μL ST1 + 200 μL ddH_2O	1.6 mM
ST3	750 μL ST2 + 250 μL ddH_2O	1.2 mM
ST4	600 μL ST3 + 300 μL ddH_2O	0.8 mM
ST5	500 μL ST4 + 500 μL ddH_2O	0.4 mM
ST6	500 μL ST5 + 500 μL ddH_2O	0.2 mM
ST7	500 μL ST6+ 500 μL ddH_2O	0.1 mM (fixed point)
Blank	500 μL ddH_2O	0 mM

2. Preparation of control samples:

a. To ensure the accuracy of the results, it is recommended that each sample be tested three times in parallel.

b. Spiked recovery (ERC) Use ERC to evaluate the authenticity and accuracy of glycerol detection, and use ERC to evaluate the validation of analytical methods and system performance. The calculation formula for the standard recovery rate = (detection value - background value) / amount of glycerin added * 100%.

Assay Procedure

1. The kit needs to be stored at -18°C or below when unopened; fresh reaction solution needs to be prepared for each reaction. Assay Buffer 2 and Enzyme Mix cannot be used again after repeated freezing and thawing twice. During the preparation of the reaction solution, Assay Buffer 2 and Enzyme Mix need to be

kept on ice. The reaction system is as follows:

	Assay Buffer I	Assay Buffer II	Enzyme Mix
Volume (μL)	68	2	10

2. Add 20 μL of the sample to be tested and the glycerin standard working solution into the corresponding wells of the 96-well transparent flat-bottomed plate. Three duplicate wells must be set up.
3. Prepare the reaction mixture according to the table. Add 80 μL of the reaction mixture to each reaction well and incubate in a 37°C constant-temperature incubator for 30 minutes. The absorbance value at 340 nm was then measured using a microplate reader.

Interpretation Of Results

1. Calculation of A340 absorbance value

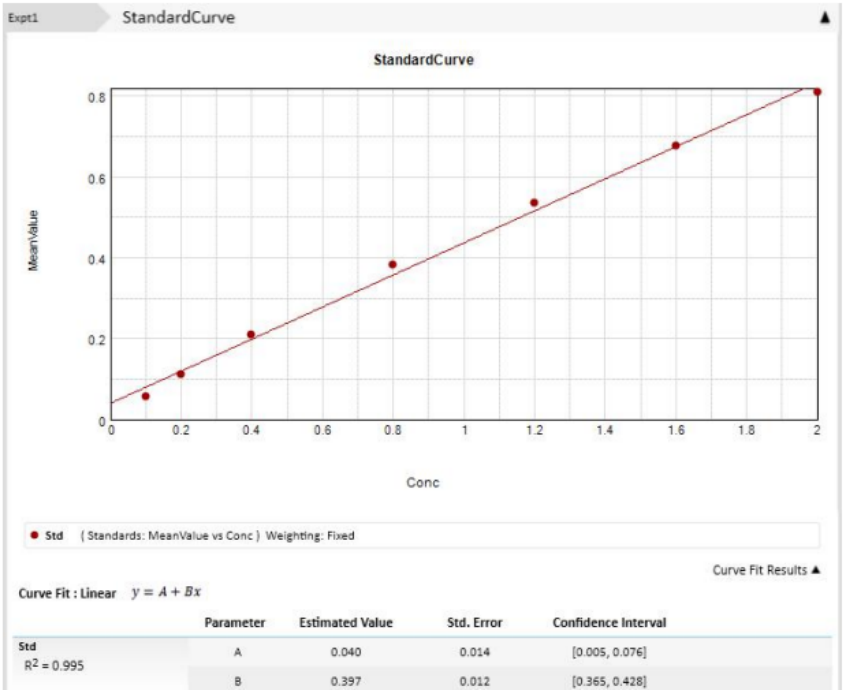
First, use the absorbance value of 0 mM concentration to calibrate the remaining standard concentration and the absorbance value of the sample to be tested.

2. Drawing and calculation of standard curve

Taking the A340 average absorbance value of each well of the standard as the ordinate (y) and the corresponding glycerol standard concentration (mM) as the abscissa (x), a linear regression equation is obtained. The fitting range of the standard curve is ST1~ST7, and the value of 0 is not included in the standard music.

That is: y=A+Bx;

The linear correlation coefficient of the standard curve is required to be $R^2 \geq 0.980$.



Substitute the absorbance value of the sample into the standard curve, read the concentration corresponding

to the sample from the standard curve, and multiply it by the corresponding dilution factor to get the actual residual amount of glycerol in the sample.

You can use the software that comes with the microplate reader as the fitting software for the calibration curve. If not, it is recommended to use professional music marking software, such as Curve Expert, ELISA Calc, etc.

Precision

Repeatability CV≤15%

Detection Limit

Limit of quantification: 0.2 mM;

Linearity

Effective linear range: 0.2 mM~2.0 mM, correlation coefficient $R^2 \geq 0.980$;

Recovery

80%~120%