Proteinase K Assay [Colorimetric]

(Cat. # 786-1807)

INTRODUCTION

Proteinase K Assay [Colorimetric] is designed for rapid and reproducible quantification of Proteinase K activity.

Proteinase K is a broad-spectrum serine protease which is routinely used to digest proteins and remove contamination from nucleic acid preparations. Proteinase K inactivates nucleases that could degrade DNA or RNA during isolation and purification.

Proteinase K Assay [Colorimetric] is based on hydrolysis of denatured hemoglobin by proteinase K which releases Folin positive amino acids and peptides. The amino acids and peptide react with Folin's reagent to give colored product which is measured at absorbance of 578nm. The linear detection limit of the assay falls in range 5 µg/ml- 100 µg/ml.

Proteinase K Assay [Colorimetric] has sufficient reagents for 100 standard test tube assays.

ITEM(S) SUPPLIED (Cat. # 786-1807)

Description	Size (100 assay)
Reagent-Hb	130 ml
Proteinase K [Standard]	10 mg
Folin's Reagent	35 ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit components as indicated on the label, upon arrival.

ITEMS NEEDED BUT NOT SUPPLIED

- TCA solution [5%] (Cat. # 786-886)
- 0.5N NaOH (Cat. # R007)
- PBS or any other diluent buffer

IMPORTANT INFORMATION

- Reagent-Hb provided in the kit can be stored at 4°C for 2-3 months. For long term storage make single use aliquots and store at -20°C.
- If TCA solution is bought from G-Biosciences (Cat. #786-886), it should be diluted 1:1 with deionized water to 5% concentration.
- If NaOH is bought from G-Biosciences (Cat. # R007) then dilute it with deionized water in ratio 1:3 (eg: to 10 ml of 2 N NaOH add 30 ml deionized water to get 0.5 N NaOH).
- Run standards with every experiment for accurate estimation.

PROTOCOL

Preparation of Proteinase K Standard curve:

1. Weigh 1 mg of Proteinase K and dissolve in 1 ml PBS.
2. Prepare the standard as below.

Tube	Proteinase K (1 mg/ml) (µl)	PBS or diluent buffer (µl)	Proteinase K concentration (µg/ml)
A	0	500	0
B	10	490	20
C	20	480	40
D	30	470	60
E	40	460	80

NOTE: *The detection limit of the assay falls in range 0.5 µg- 10 µg (5µg/ml -100 µg/ml) (Fig1). This is just an example. The end user can make own standard graph as per requirement.*

Protocol for standard tube assay:

1. Bring the kit components to room temperature.
2. Label the test tubes (5-8ml) for Blank, standard and test sample. Add 1.25 ml of Reagent-Hb per tube.
3. Incubate the tubes at 37°C for 5 minutes.
4. Add 100 µl of sample or standard per tube. Add 100 µl PBS or diluent buffer to the blank tube.
5. Incubate the tubes at 37°C for 10 minutes.
6. Add 2.5 ml of 5% TCA to tubes to stop reaction. Close the cap and briefly vortex.
7. Transfer 1 ml of suspension from tubes to microfuge tubes. Centrifuge at 15000g for five minutes.
8. Transfer 0.5 ml supernatants from microfuge tubes to test tubes.
9. Add 1 ml of 0.5 N NaOH to each tube and briefly vortex to mix.
10. Add 0.3 ml of Folin's reagent to each tube and briefly vortex to mix.
11. Incubate the tubes at room temperature for 30 minutes.
12. Measure OD at 578 nm and plot the graph for standard to determine Proteinase K in unknown samples.

Protocol for standard microplate or microwell assay:

1. After the TCA treatment and centrifugation step (see Protocol for standard tube assay) transfer 50 µl of supernatants from tubes to microtiter plate wells.
2. Add 100 µl of 0.5 N NaOH to each well with help of multichannel pipette and mix well.
3. Add 30 µl of Folin's reagent to each well with help of multichannel pipette and mix well with pipette.
4. Incubate the tubes at room temperature for 30 minutes.
5. Measure OD at 578 nm and plot the graph for standard to determine Proteinase K in unknown samples.

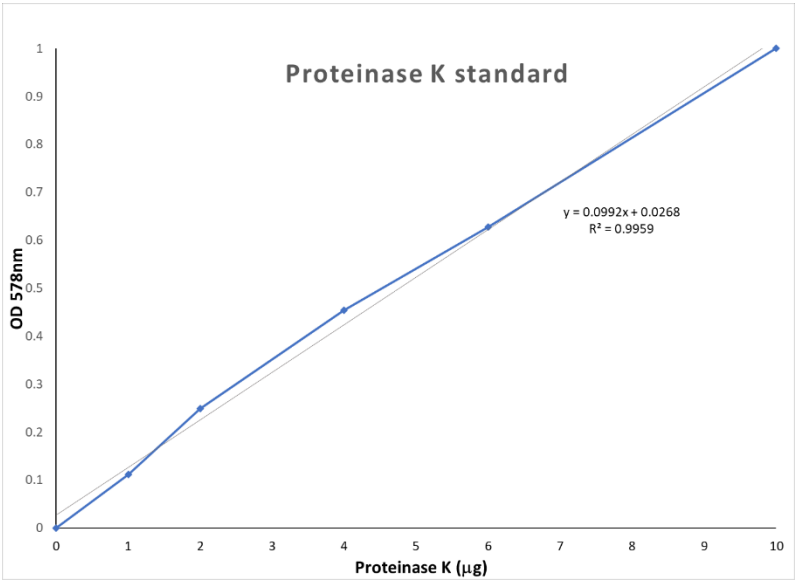


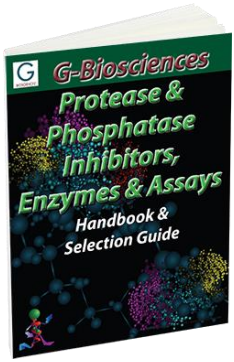
Fig.1

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
The absorbance value of test sample falls above linear range	Sample has high concentration of proteinase K	Dilute the sample.
Absorbance values for standard coming lower than expected.	Reagent-Hb not stored properly	Store Reagent-Hb as mentioned in protocol

RELATED PRODUCTS

Download our Protease & Phosphatase Inhibitors, Enzyme & Assays Handbook.



http://info.gbiosciences.com/protease-phosphatase-inhibitors-enzymes-assay-handbook

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