Neutral Protease Microplate Assay Kit

User Manual

Catalog # CAK1078

(Version 1.4E)

Detection and Quantification of Neutral Protease (NP) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Neutral Protease is frequently used as a secondary enzyme in conjunction with collagenase and/or other proteases in many primary cell isolation and tissue dissociation applications. Neutral Protease dissociates fibroblast-like cells more efficiently than epithelial-like cells so it has also been used for differential isolation and culture applications. Other advantages are its non-mammalian (bacterial) source and its ability to be inhibited by EDTA.

The assay is initiated with the enzymatic catalysis of casein by NP in neutral environment. The enzyme catalysed reaction products can be measured at a colorimetric readout at 660 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Stop Solution	12 ml x 1	4 °C
Reaction Buffer	6 ml x 1	4 °C
Dye Reagent	4 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Standard Diluent	5 ml x 1	4 °C
Positive Control	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate: add 4 ml Assay Buffer to dissolve before use.

Standard: add 1 ml Standard Diluent to dissolve before use, mix, heat at 50 °C water bath; then add 500 µl into 500 µl Standard Diluent, the concentration will be 5 mmol/L.

Positive Control: add 100 µl Assay Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Positive Control	Standard	Blank
Sample	40 µl	--	--	--	--
Positive Control	--	--	40 µl	--	--
Standard	--	--	--	40 µl	--
Standard Diluent	--	--	--	--	40 µl
Assay Buffer	--	40 µl	--	40 µl	40 µl
Substrate	40 µl	40 µl	40 µl	--	--
Mix, put it in the oven, 40 °C for 15 minutes.					
Stop Solution	120 µl	120 µl	120 µl	120 µl	120 µl
Mix, centrifuged at 10,000g 4 °C for 10 minutes, add the supernatant into the microplate.					
Supernatant	100 µl	100 µl	100 µl	100 µl	100 µl
Reaction Buffer	60 µl	60 µl	60 µl	60 µl	60 µl
Dye Reagent	40 µl	40 µl	40 µl	40 µl	40 µl
Mix, incubate at room temperature for 20 minutes, measured at 660 nm and record the absorbance.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) If there is any precipitation or floc before read out, please centrifuged it in the microcentrifuge tubes at 4000g for 5 minutes, then add the supernatant into the plate, measured at 660 nm.

4) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of NP activity is the enzyme that generates 1 μmol of Tyrosine per minute.

1. According to the protein concentration of sample

$$NP\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / C_{Protein}$$

2. According to the weight of sample

$$NP\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / W$$

3. According to the quantity of cells or bacteria

$$NP\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / N$$

4. According to the volume of serum or plasma

$$NP\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control}) / V_{Sample} / T$$

$$= 0.333 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Control})$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 μmol/ml;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Sample}: the volume of sample, 0.04 ml;

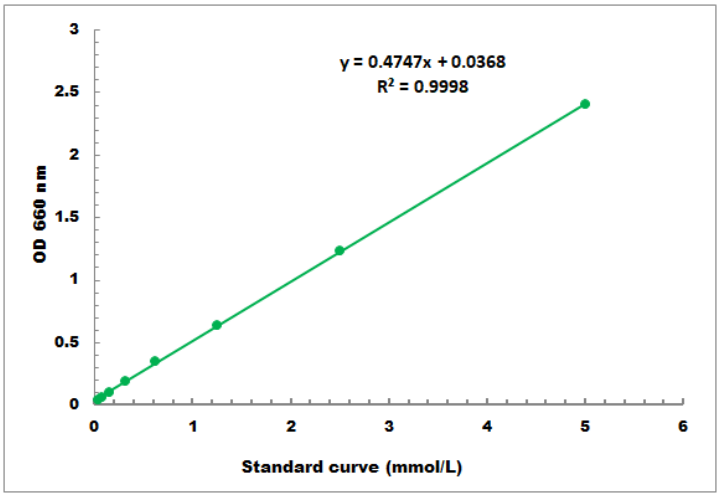
$V_{Standard}$: the volume of standard, 0.04 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

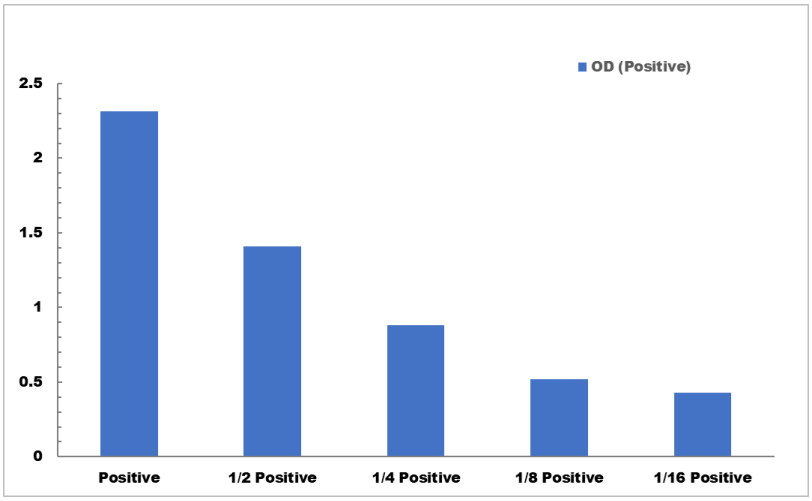
T: the reaction time, 15 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.05 mmol/L - 5 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES