Hyaluronidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1279

(Version 1.1A)

Detection and Quantification of Hyaluronidase Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Hyaluronidase catalyzes the depolymerization of mucopolysaccharides, hyaluronic acid, and the chondroitin sulfates A and C. The enzyme is widely distributed in animal tissues but is found in great concentrations in the bovine and ovine testes. It is also produced by a number of bacteria. Hyaluronidase from bovine testes has a molecular weight of 55,000. Purified hyaluronidase is used clinically for the intradermal administration of large volumes of fluid when intravenous injections are contraindicated. The enzyme is administered prior to or simultaneously with the fluid and it facilitates absorption of the fluid.

Hyaluronidase Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring hyaluronidase activity in a variety of samples. The hyaluronidase reacts with substrate and liberates N-acetylglucosamine. N-acetylglucosamine reacts with p-dimethylamino-benzaldehyde to form a colored complex, which is measured spectrophotometrically at 530 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Stop Solution A	5 ml x 1	4 °C
Stop Solution B	0.35 ml x 1	4 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	8 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C, keep in dark
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 530 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Boiling water bath

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 20 mmol/L of standard top solution, store at 4 °C for 1-2 months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625/0.312 mmol/L.

Substrate: Add 6 ml Dilution and heat at 50 °C for 20 minutes to dissolve completely before use. Store at 4 °C for 1-2 weeks or -20 °C for 1-2 months after reconstitution.

Stop Working Solution: Briefly centrifuge Stop Solution B prior to opening. Transfer 0.35 ml Stop Solution B to Stop Solution A and mix thoroughly before use. Keep in dark or store at 4 °C for 1 week or -20 °C for 1 month after reconstitution.

Dye Reagent: Add 8 ml Dye Reagent Diluent to dissolve before use. Keep in dark or store at 4 °C for 1 weeks or -20 °C for 1 month after reconstitution. Heat to dissolve completely if crystals formed.

Positive Control: Briefly centrifuge prior to opening. Add 0.5 ml Diluent to dissolve before use. Store at 4 °C for 1-2 weeks or -20 °C for 3 months after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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 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

Detection directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tube:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Sample	20 µl	--	--	--	--
Assay Buffer	--	20 µl	--	--	--
Positive Control	--	--	20 µl	--	--
Substrate	60 µl	60 µl	60 µl	--	--
Mix in centrifuge tube, put it in the oven at 37 °C for 45 minutes.					
Standard	--	--	--	80 µl	--
Distilled water	--	--	--	--	80 µl
Stop Working Solution	50 µl	50 µl	50 µl	50 µl	50 µl
Put it in the boiling water for 15 minutes. Cool and transfer into the microplate, then add Dye Reagent.					
Dye Reagent	70 µl	70 µl	70 µl	70 µl	70 µl
Mix by pipetting several times, put it in the oven, 37 °C for 5 minutes, record absorbance measured at 530 nm.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background

control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of hyaluronidase activity is defined as the enzyme liberates 1 μmol N-acetylglucosamine per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Control}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{Standard}}{V_{Sample}} \times n \quad (U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T} \quad (U/10^4)$$

2.2 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T} \quad (U/g)$$

2.3 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T} \quad (U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

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

$V_{Standard}$: the volume of standard in assay procedure, mL

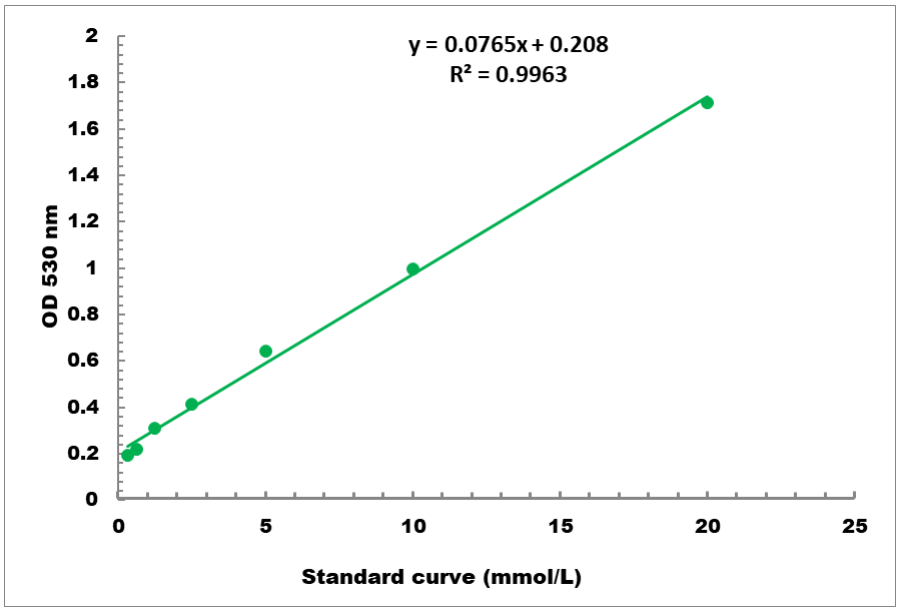
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

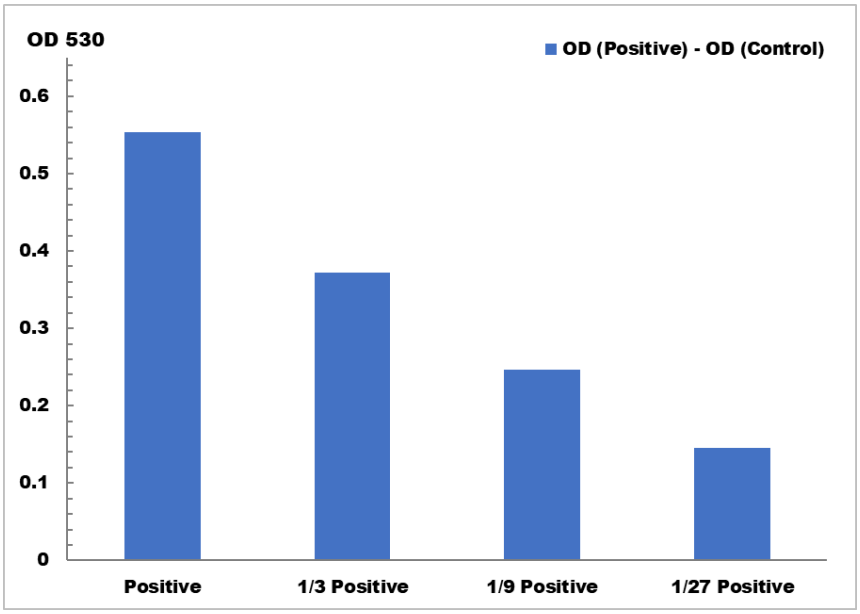
T: the reaction time, 45 minutes

VIII. TYPICAL DATA

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



Detection Range: 0.2 mmol/L - 20 mmol/L



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