Hyaluronic Acid

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1280

(Version 1.1A)

Detection and Quantification of Hyaluronic Acid (HA) Content in Fermentation broth, Tissue extracts, Cell extracts, Cell culture media and Other biological fluids

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

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I. INTRODUCTION

Hyaluronic acid (HA) is a highly polymerized mucopolysaccharide present in the highest concentrations in mammals in vitreous humor of the eye, human umbilical cords and joint fluids. It is one of the constituents of ground substance. It may be isolated in widely ranging degrees of purity and polymerization. Hyaluronic acid consists of a polymer of N-acetylglucosamine and glucuronic acid probably arranged as alternating units in a flexible chain. The HA molecule is negatively charged but the degree of charge varies greatly with the ionic environment. This negative charge results in the mutual precipitation of cationic proteins such as albumin at low pH as in the Hyaluronidase assay procedure.

Hyaluronic Acid Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring Hyaluronic Acid content in a variety of samples. The hyaluronidase reacts with hyaluronic acid 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	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
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
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. Cold-ethanol
4. 20 mg/mL proteinase K
5. Pipettor, multi-channel pipettor
6. Pipette tips
7. Mortar
8. Centrifuge
9. Timer
10. Boiling water bath

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml Diluent and heat at 50 °C for 20 minutes to dissolve completely to generate 2.5 mg/mL of standard top solution, store at 4 °C for 1-2 weeks or -20 °C for 1-2 months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using Diluent to make the standard curve. The concentration of standard curve could be 2.5/1.25/0.625/0.312/ 0.156/0.078/0.039 mg/mL.

Enzyme: Add 6 ml Diluent to dissolve before use. Store at 4 °C for 1-2 weeks or -20 °C for 3 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.

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

V. SAMPLE PREPARATION

1. For Fermentation broth samples

Centrifuged Fermentation broth at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube. Adjust the conductivity to approximately 20-30 mS/cm by adding KCl or NaCl. Add 4 volumes of cold-ethanol followed by incubation at -20 °C for 2-4 hours for precipitation. Centrifuged at 10,000g 4 °C for 10 minutes and wash the pellet with 1 ml cold-70% ethanol by centrifugation, followed by air-drying the pellet. Dissolve the pellet with 50-100 µl distilled water 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, add 10 µl 20 mg/mL proteinase K (final concentration 100-200 µg/mL) and incubate at 55-60 °C for 3-6 hours (or overnight for tough tissues like cartilage/skin). Inactivate proteinase K by heating at 80 °C for 15-20 min. Centrifuge at 10,000g 4 °C for 10 min. Collect the supernatant for detection.

3. For cell samples for measurement of intracellular and/or cell-surface bound HA

Collect cell into centrifuge tube, discard the supernatant after centrifugation, homogenize with 1 ml Assay Buffer on ice for 5×10^6 cell, add 10 µl 20 mg/mL proteinase K (final concentration 100-200 µg/mL) and incubate at 55-60 °C for 3-6 hours. Inactivate proteinase K by heating at 80 °C for 15-20 min. Centrifuge at 10,000g 4 °C for 10 min. Collect the supernatant for detection.

4. For cell culture supernatant samples for measurement of secreted HA

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tube:

Reagent*	Sample**	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Enzyme	60 µl	60 µl	60 µl
Mix in centrifuge tube, put it in the oven at 37 °C for 60 minutes.			
Stop Working Solution	50 µl	50 µl	50 µl
Put it into the boiling water for 15 minutes. Cool and transfer into the microplate, then add Dye Reagent.			
Dye Reagent	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:

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**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

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \quad (mg/ml)$$

Calculate the initial concentration according to sample preparation procedure.

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

2.2 According to the quantity of cells

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \quad (mg/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \quad (mg/g)$$

2.4 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \quad (mg/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mg/ml

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

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

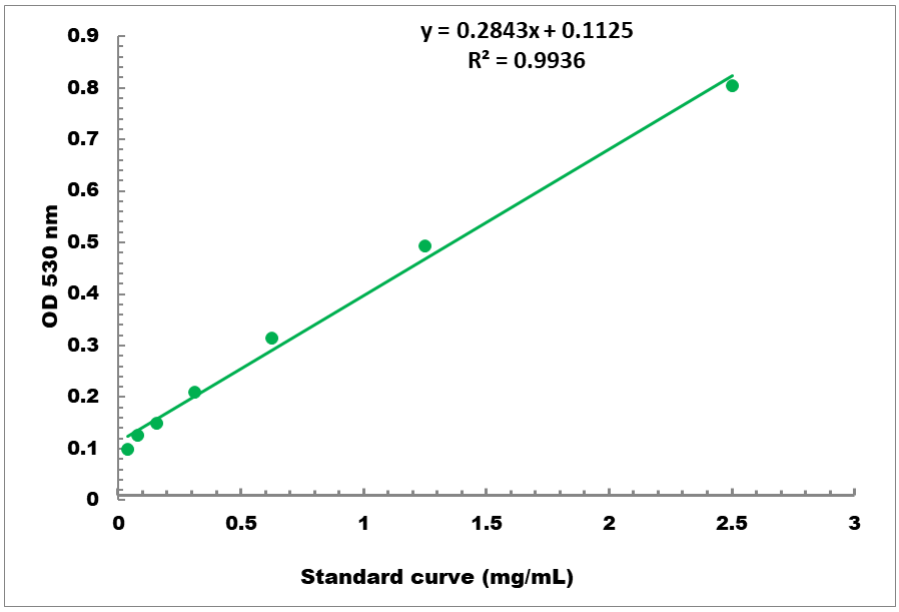
V_{Assay}: the volume of Assay Buffer, µl

W: the weight of sample, g

N: the quantity of cells, 10^4

VIII. TYPICAL DATA

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



Detection Range: 0.025 mg/mL - 2.5 mg/mL