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Hyaluronidase Enzymatic Assay Kit

Cat No: KBBA05

Ver3.1

RUO

Enzymatic Assay for Quantification of Hyaluronidase activity in cell culture supernatant and pharmaceutical preparations

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBBA05

100 assays



KRISHGEN BioSystems

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Introduction:

Hyaluronidase is a family of enzymes that degrade hyaluronic acid by catalyzing the hydrolysis of hyaluronan, a constituent of the interstitial barrier. Hyaluronidase lowers the viscosity of hyaluronan, thereby increasing tissue permeability. It is, therefore, used in medicine in conjunction with other drugs to speed their dispersion and delivery.

Intended Use:

The Hyaluronidase Assay kit is intended for determination of Hyaluronidase enzyme activity in cell culture supernatant and pharmaceutical preparations.

Principle:

The Hyaluronidase Assay kit uses a two-step turbidimetric reaction to measure hyaluronidase activity by the amount of hyaluronic acid that is hydrolyzed. A stop reagent halts the enzymatic reaction and forms turbidity with any residual hyaluronic acid in the well. The decrease in turbidity at 600 nm is directly proportional to hyaluronidase activity in the sample.

Materials Provided:

1. Microtitre Plate, uncoated (12x8) - 1 no
2. Hyaluronic Acid (HA) Substrate - 1.5 ml
3. Hyaluronidase Enzyme Standard (purified from bovine testes, lyophilized) - 2 mg
4. Hyaluronidase Enzyme Buffer - 10 ml
5. Assay Buffer - 5 ml
6. Stop Solution - 20 ml
7. Instruction manual

Materials Not Provided:

1. Spectrophotometer/ Plate Reader with 600nm filter.
2. Pipettes ranging from 25 ul - 1000 ul
3. Test tubes
4. Deionized or distilled water
5. Graph Paper

Storage Information:

1. Upon receipt store the Hyaluronidase Enzyme at 2-8°C and other components at -20°C.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation (all reagents should be prepared freshly prior to use):

The Hyaluronidase Assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Reagent Preparation: Prior to assay, equilibrate all components to room temperature.

Working Reagent: Working Reagent should be prepared fresh and used within two hours. Prepare enough Working Reagent for each well by combining **10 ul HA Substrate** and **35 ul Assay Buffer**.

Hyaluronidase Standard Preparation: Hyaluronidase should be prepared in Enzyme Buffer and used fresh. Albumin and other proteins interfere with this assay and should not be included in the Enzyme Buffer.

Reconstitute the 2 mg Hyaluronidase vial with 2 ml Enzyme Buffer to generate a 120 U/ml concentration. Keep the vial for 5 mins with gentle agitation before making further dilutions. It is recommended to make aliquots of 200 ul of this main stock and store at -20°C for future use.

Prepare the Standards stock by diluting the 200 ul aliquot solution further as per the below table. Thus the Hyaluronidase Standard concentrations are 14 U/ml, 7 U/ml, 3.5 U/ml and Enzyme Buffer serves as the zero standard (0 U/ml).

Standard Concentration	Standard No	Dilution Particulars
120 U/ml	Reconstituted Main Stock	Standard provided in the kit (2mg) + 2 ml Enzyme Buffer
28 U/ml	Diluted Main Stock	9.34 ul Aliquoted Main Stock + 30.66 ul Enzyme Buffer
14 U/ml	Standard No.4	20 ul Standard No.5 + 20 ul Enzyme Buffer
7 U/ml	Standard No.3	20 ul Standard No.4 + 20 ul Enzyme Buffer
3.5 U/ml	Standard No.2	20 ul Standard No.3 + 20 ul Enzyme Buffer
0 U/ml	Standard No.1	40 ul Enzyme Buffer

Assay Procedure:

1. Pipette **40 ul** of **Enzyme Buffer** into blank well
2. Add **20 ul** of **Standards** or **Samples** to respective wells.
3. Incubate for 15 min at Room Temperature.
4. Add **40 ul Working Reagent** to all wells and tap plate to mix thoroughly.
5. Incubate for 45 minutes at Room Temperature.
6. Add **160 ul Stop Solution** to each well. Tap plate to mix well.
7. Incubate for 10 minutes at room temperature.
8. Read Abs at 600 nm.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using Standard graph paper, plot the average value (absorbance 600nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points.

To determine the unknown Hyaluronidase activity concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Hyaluronidase activity Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

Note:

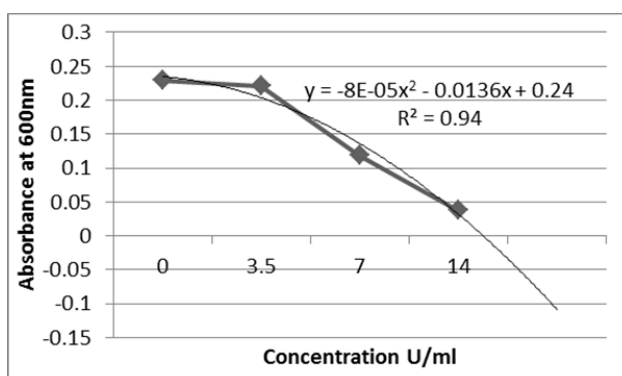
It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 14 U/ml standard.

Typical Data

Hase Std U/ml	Abs A	Abs B	Mean Abs
0	0.2264	0.2319	0.229
3.5	0.2219	0.2196	0.221
7	0.1271	0.1099	0.119
14	0.0388	0.0377	0.038

Typical Graph



Precautions:

Do not mix reagents from different kits or lots. Reagents from different manufacturers should not be used with this kit.

Performance Characteristics:

Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard less 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be 2 U/ml.

Precision:

Intra-Assay: CV<5%

Inter-Assay: CV<8%

Safety Precautions:

- **This kit is For Research Use only.** Follow the working instructions carefully.

- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves
 - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



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