KRISHZYME® Hyaluronidase Enzymatic Assay Kit

REF KBBA05

Ver 5.2

RUO

Enzymatic Assay for Quantification of Hyaluronidase activity in cell culture supernatant and other biological fluids

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 **Σ 200 Tests**

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Introduction:

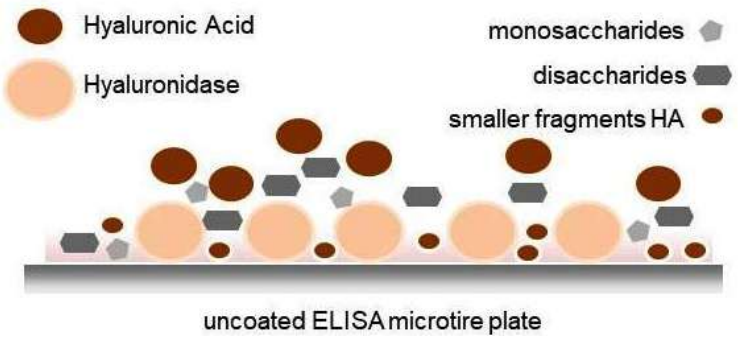
Hyaluronidase is a family of enzymes that degrade hyaluronic acid by catalyzing the hydrolysis of hyaluronan, a constituent of the interstitial barrier. Hyaluronidase lowers the viscosity of hyaluronan, thereby increasing tissue permeability. It is, therefore, used in medicine in conjunction with other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

Intended Use:

The KRISHZYME® Hyaluronidase Assay kit is intended for determination of Hyaluronidase enzyme activity in cell culture supernatant and other biological fluids.

Principle:

The KRISHZYME® Hyaluronidase Assay kit uses a turbidimetric reaction to measure hyaluronidase activity by the amount of hyaluronic acid that is hydrolyzed in a microliter format. A stop reagent halts the enzymatic reaction and forms turbidity with any residual hyaluronic acid in the well. The decrease in turbidity at 630 nm is directly proportional to hyaluronidase activity in the sample.



Materials Provided:

1. Microtiter Plate, uncoated (12 x 8 wells) - 2 no
2. Hyaluronic Acid (HA) Substrate - 1450 ul
3. Hyaluronidase Standard lyophilized (1400 U/ml) – 2 vial
4. Enzyme Buffer - 20 ml
5. Assay Buffer - 15 ml
6. Stop Solution - 35 ml
7. Instruction Manual

Materials Not Provided:

1. Spectrophotometer/ Plate Reader with 630 nm filter.
2. Pipettes ranging from 25 ul to1000 ul.
3. Test tubes.
4. Deionized or distilled water.
5. Graph Paper.

Storage Information:

1. Upon receipt store all the components at 2-8°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 KRISHZYME® 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 pipette is recommended.

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 **7.2 ul HA Substrate** with **42.8 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 lyophilized Hyaluronidase enzyme vial with 100 ul Enzyme Buffer to generate a 1400 U/ml concentration. Keep the vial for 5 mins with gentle agitation before making further dilutions. It is recommended to make aliquots of this main stock and store at -20ºC for future use.

Prepare the Standards stock by diluting the reconstituted stock solution further as per the below table. Thus the Hyaluronidase Standard concentrations are 896 U/ml, 448 U/ml, 224 U/ml, 112 U/ml, 56 U/ml, 28 U/ml and Enzyme Buffer serves as the zero standard (0 U/ml).

Standard Concentration	Standard No	Dilution Particulars
1400 U/ml	Reconstituted Standard	Lyophilized Standard + 100 ul Enzyme buffer
896 U/ml	Standard No.7	64 ul Reconstituted Standard + 36 ul Enzyme Buffer
448 U/ml	Standard No.6	50 ul Standard No.7 + 50 ul Enzyme Buffer
224 U/ml	Standard No.5	50 ul Standard No.6 + 50 ul Enzyme Buffer
112 U/ml	Standard No.4	50 ul Standard No.5 + 50 ul Enzyme Buffer
56 U/ml	Standard No.3	50 ul Standard No.4 + 50 ul Enzyme Buffer
28 U/ml	Standard No.2	50 ul Standard No.3 + 50 ul Enzyme Buffer
0 U/ml	Standard No.1	Only Enzyme Buffer

Assay Procedure:

1. Pipette **40 ul** of **Enzyme Buffer** into blank well.

2. Add **20 ul** of prepared **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 Absorbance at 630 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 630 nm) 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.

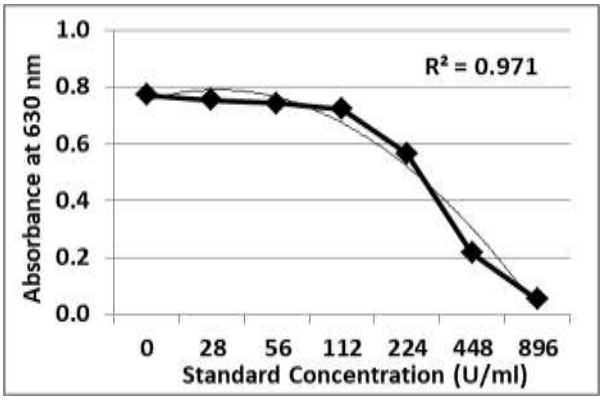
Software which is able to generate a cubic spline curve-fit or 4-PL (2nd order) is best recommended for automated results.

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 896 U/ml standard.

Typical Data (representative only)

Hyaluronidase Activity Concentration (U/ml)	Mean Absorbance at 630 nm	Interpolated Activity Concentration	% Interpolated Activity Concentration against Actual activity Concentration
0	0.772	--	--
28	0.753	33.6	120.0
56	0.741	65.8	117.5
112	0.722	114.6	102.3
224	0.566	222.8	99.5
448	0.216	449.6	100.4
896	0.053	889.3	99.3

Typical Graph (representative only)



Precautions:

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

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilution linearity assay to assure quality results.

For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

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 ~20 U/ml.

Assay Range:
0 U/ml to 896 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/v) 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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