Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Hyaluronidase Activity ELISA Kit

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(Catalog # E4909-100; 100 assays, Store kit at -20 °C)

I. Introduction:

Hyaluronidases, also known as hyaluronoglucosidases (HYALs) are family of enzymes that catalyse the degradation of hyaluronic acid (HA) by cleaving its glycosidic bonds. HA is a nonsulfated glycosaminoglycan and a major component of the extracellular matrix. It is involved in many biological processes including structural support, cell migration and tissue turnover. In humans, there are six known HYALs and are present in organs including spleen, skin, eyes, liver, kidneys, uterus, placenta etc. and in body fluids including saliva, tears, blood, semen etc. They increase membrane permeability, and make tissues more readily permeable. However, HYALs have been also implicated in the progression of many solid tumor types including colorectal, bladder, prostate, breast and brain cancer. Additionally, HYALs have been used in ophthalmic surgery and dermatology. **BioVision's Hyaluronidase Activity ELISA Kit** is a high throughput, quantitative immunoassay desgined to determine the activity of HYALs in various biological samples. In contrast to the traditional turbidity assay, this kit offers a simple, sensitive and convenient method to measure the activity of HYALs in biological samples. In this assay, samples are added to the wells of a 96-well plate pre-coated with biotin-HA. If the sample has any HYAL activity, it will bind to the biotin-HA on the plate and thus less free biotin-HA sites will be available following washing. The biotin-HA is then detected using a specific streptavidin probe measured at OD 450 nm. The absorbance signal is inversely proportional to the HYAL activity in samples. The kit can detect as low as 0.5 mU of HYAL activity under the assay conditions.

II. Application:

- Determination of hyaluronidase activity in various biological samples.

III. Sample Types:

- Body fluids (i.e. saliva and urine)
- Tissue sample (i.e. brain)

IV. Kit Contents:

Components	E4909-100	Cap Code	Part Number
Pre-coated 96 Well Strip Plate	8 x 12 Strips	--	E4909-100-1
HYAL Assay Buffer	40 ml	WM	E4909-100-2
HYAL Standard	1 vial	Yellow	E4909-100-3
HRP Conjugate	1 vial	Blue	E4909-100-4
TMB Substrate	10 ml	Amber	E4909-100-5
Stop Solution	10 ml	Blue	E4909-100-6
Wash Buffer (10X)	50 ml	NM	E4909-100-7
Conjugate Buffer	40 ml	Green/NM	E4909-100-8
Plate Sealers	4	--	E4909-100-9

V. User Supplied Reagents and Equipment:

- Dounce Tissue Homogenizer (BioVision Cat No. 1998)
- Multi-well spectrophotometer
- Multichannel pipette
- Microcentrifuge tube.

VI. Storage Conditions and Reagent Preparations:

Store the kit at -20 °C, protected from light. Briefly centrifuge all small vials at a low speed prior to opening. The kit components are stable for one year, when stored as recommended. Read the entire protocol before performing the experiment.

- **Pre-Coated 96-well Strip Plate:** Do not open until ready to use. Bring to room temperature (RT) prior to opening. After opening, immediately store the remaining unused Pre-coated strips at 4 °C in a foil bag with desiccant to protect the strip wells from moisture.
- **HYAL Assay Buffer, Stop Solution & Conjugate Buffer:** Ready to use. Warm to RT before use. Store at 4 °C.
- **HYAL Standard:** Briefly centrifuge before opening the tube. Reconstitute the vial in 0.5 ml of HYAL Assay Buffer to prepare the Stock HYAL Standard solution (100 U/ml). Divide into aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles.
- **HRP Conjugate:** Briefly centrifuge before opening the tube. Reconstitute the vial in 1 ml Conjugate Buffer to prepare the stock HRP Conjugate. Prepare the HRP Conjugate working solution by diluting the stock HRP Conjugate 200 fold (i.e. mix 20 µl stock HRP Conjugate with 4 ml Conjugate Buffer). The HRP Conjugate working solution is stable for 2 weeks at 4 °C.
- **TMB Substrate:** Ready-to-use solution. Bring to RT prior to use. Store at 4 °C, protected from light.
- **Wash Buffer (10X):** Warm to RT prior to use. If crystals are present, mix gently until the crystals are completely dissolved. Prepare 100 ml 1X Wash Buffer by mixing 10 ml Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored for one month at 4 °C.

VII. Hyaluronidase Activity Assay Protocol:

1. Sample Preparation:

Saliva / Urine:

1. Centrifuge 0.4 ml of sample at 10,000 x *g* and 4 °C for 10 min. Transfer 0.2 ml of supernatant in a clean microcentrifuge tube.
2. Add 0.2 ml of HYAL Assay Buffer and mix.
3. Use 100 µl per well for the assay.

Tissue Lysates:

1. Cut, weigh and transfer ~100 mg of tissue into a microcentrifuge tube.
2. Homogenize the tissue in 0.4 ml of cold HYAL Assay Buffer using Dounce Tissue Homogenizer (BV Cat# 1998) for 5-10 min.
3. Centrifuge the sample at 10,000 x *g* for 20 min at 4 °C. Transfer the supernatant into a clean microcentrifuge tube.
4. Mix 0.2 ml of supernatant with 0.2 ml of HYAL Assay Buffer.
5. Use 100 µl per well for the assay.

2. HYAL Standard Preparation:

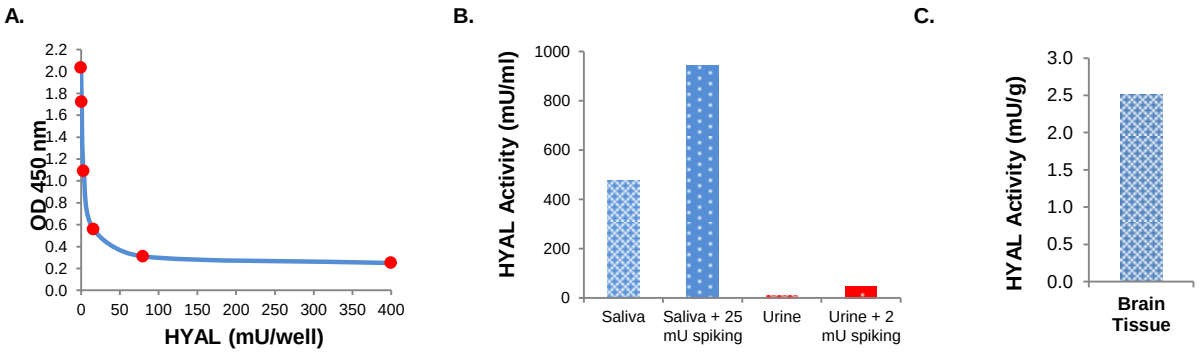
a. Dilute 20 µl of stock HYAL Standard (100 U/ml) with 480 µl of HYAL Assay Buffer (1:25 dilution) to prepare 4 U/ml (4000 mU/ml) HYAL Standard solution and mix well.
b. Perform a 5-fold serial dilution sequentially using 4 U/ml HYAL Standard solution. i.e. mix 0.2 ml of 4 U/ml HYAL Standard with 0.8 ml of HYAL Assay Buffer to prepare S4 Standard solution (800 mU/ml). Then mix 0.2 ml of 800 mU/ml HYAL Standard with 0.8 ml of HYAL Assay Buffer to prepare S3 Standard solution (160 mU/ml). Then mix 0.2 ml of 160 mU/ml Standard with 0.8 ml of HYAL Assay Buffer to prepare HYAL S2 Standard solution (32 mU/ml). Then mix 0.2 ml of 3.2 U/ml HYAL Standard with 0.8 ml of HYAL Assay Buffer to prepare S1 Standard solution (6.4 mU/ml). **S0 Standard** is HYAL Assay Buffer only. **Notes:** Diluted HYAL Standards are stable for up to 2 weeks at -20 °C. A Standard Curve must be run with each assay.

HYAL Standard	S0	S1	S2	S3	S4	S5
Activity (mU/well)	0	0.64	3.2	16	80	400

3. Add 100 µl of each **Standard solution (S0-S5)** into the appropriate wells to prepare 0, 0.64, 3.2, 16, 80 and 400 mU/well as shown in the table above. Add 100 µl of Sample into a desired well. **Notes: a.** We recommended running all Standards and sample(s) in duplicates. **b.** Prepare all reagents, Standards and sample(s) as instructed. **c.** Prepare enough reagents for the number of assays to be performed.
4. Cover the plate with a plate sealer and incubate at 37 °C for 2 hr. **Note:** For samples with low HYAL activities, longer incubation time of 8-12 hr and further dilution of the HYAL Standards is recommended.
5. Aspirate the solution from each well and wash each well 5 times. Wash by filling each well with 250 µl of 1X Wash Buffer and incubating for 30 sec. Remove the 1X Wash Buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times. **Note:** Complete removal of Wash Buffer is essential for accurate results.
6. Add 50 µl of **HRP Conjugate working solution** to each well. Mix well and cover with a plate sealer and incubate the plate for 30 min at RT with gentle orbital shaking.
7. Aspirate the solution from each well and wash each well 5 times. Wash as mentioned in step 5. **Note:** Complete removal of Wash Buffer is essential for accurate results.
8. Remove any residual Wash Buffer from the wells and add 100 µl **TMB Substrate** to all wells. Tap or shake the plate occasionally to ensure complete mixing.
9. Measure the OD of the HYAL Standard well (S0) at 650 nm in kinetic mode. When the reading is ~1.0, add 50 µl of **Stop Solution** into each well and gently tap the plate to ensure thorough mixing.
10. Measure the absorbance in a microplate reader at 450 nm at RT within 15 min of adding Stop Solution.

VIII. Calculation:

Plot the HYAL Standard Curve as relative OD 450 nm of each HYAL Standard (Y) vs. the HYAL Activity (X). The HYAL activity in each sample is calculated from the HYAL Standard Curve. The specific enzyme activity is calculated by dividing by the sample volume (mU/ml) or mass (mU/mg). If the sample(s) are diluted, multiply with the dilution factor the determined activity in the well. **Note:** One unit is equivalent to the change of 0.007/min in the HA turbidity assay measured at 600 nm, pH 5 and 37 °C.



Figures. **A.** HYAL Standard Curve. This Standard Curve is for demonstration only. **B.** Spike recovery experiment using human saliva and urine samples before and after spiked with 25 mU and 2 mU of HYAL respectively. Spike recovery was > 85%. C. HYAL activity determined in brain tissue using the assay conditions.

IX. Related Products:

Sulfated-Glycosaminoglycans Assay Kit (K2074)

Total Glycosaminoglycans Assay Kit (Colorimetric) (K2085)

FOR RESEARCH USE ONLY! Not to be used on humans.