

Hyaluronidase Inhibitor Screening ELISA Kit

08/21

(Catalog # E4911-100; 100 assays, Store 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. Currently there are various hyaluronidase inhibitors including heparins, chitosans, trimethoprim, indomethacin etc they may serve as anti-inflammatory, anti-aging, anti-microbial or anticancer agents. **BioVision's Hyaluronidase Inhibitor Screening ELISA Kit** is a high-throughput ELISA as compared to the traditional turbidity assay for identifying HYAL inhibitors. In this assay, HYAL and test inhibitors are pre-incubated before adding into the wells of a 96-well plate, pre-coated with biotin-HA. Biotin-HA is then detected using a specific avidin-probe measured at OD 450 nm. Samples containing HYAL inhibitor will have more biotin-HA sites available, thereby resulting in higher absorbance at 450 nm as compared to samples without the HYAL inhibitor. By using different concentrations of the HYAL inhibitor, the assay can also determine the IC₅₀. The kit offers a simple, sensitive and high throughput assay to identify HYAL inhibitors as well as determine the IC₅₀ of any specific HYAL inhibitor. The HYAL inhibitor, heparin is also included in the kit.

II. Application:

- Screening of hyaluronidase inhibitors

III. Kit Contents:

Components	E4911-100	Cap Code	Part Number
Pre-coated 96 Well Strip Plate	8 X 12 Strips	--	E4911-100-1
HYAL Assay Buffer	40 ml	WM	E4911-100-2
Human SPAM1	1 vial	Orange	E4911-100-3
HRP Conjugate	1 vial	Blue	E4911-100-4
TMB Substrate	10 ml	Amber	E4911-100-5
Stop Solution	10 ml	Blue	E4911-100-6
Wash Buffer (10X)	50 ml	NM	E4911-100-7
Conjugate Buffer	40 ml	Green/NM	E4911-100-8
Heparin (1 mg/ml)	1 ml	Yellow	E4911-100-9
Plate Sealers	4	--	E4911-100-10

IV. User Supplied Reagents and Equipment:

- PBS
- Multi-well spectrophotometer (plate reader)
- Multichannel pipette

V. Storage Conditions and Reagent Preparations:

Store the kit at -20 °C. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

- **HYAL Assay Buffer, TMB Substrate, Stop Solution, Conjugate Buffer and Heparin (1 mg/ml):** Ready to use. Warm to Room Temperature (RT) before use. Store at 4 °C.
- **Human SPAM1:** Spin briefly before opening the tube. Reconstitute the vial with 220 µl of PBS to prepare the Stock Human SPAM1. Vortex briefly and store at 4 °C. **Do not store at -20 °C.**
- **HRP Conjugate:** Spin briefly before opening the tube. Reconstitute the vial with 1 ml of Conjugate Buffer to prepare stock HRP Conjugate. Dilute the stock 200X (i.e. mix 20 µl stock HRP Conjugate with 4 ml Conjugate Buffer) to prepare the HRP Conjugate working solution. The HRP Conjugate working solution is stable at 4 °C for 2 weeks.
- **Wash Buffer (10X):** Bring the bottle to RT. If crystals are present, warm up to RT and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4 °C for one month.

VI. Hyaluronidase Inhibitor Screening Assay Protocol:

Notes: We recommend running all samples in duplicates. Prepare all reagents as instructed. Calculate and mix enough reagents for the number of assays to be performed.

- Preparations of Test Compound, Solvent Control, Inhibitor Control, Enzyme Control & Background Control:** Dissolve Test Compound(s) in an appropriate solvent (i.e. DMSO) to prepare the desired concentrations. Add 2 µl of compound into an eppendorf tube labeled as **Test Compound [TC]** and 2 µl of solvent into another tube as **Solvent Control [SC]**. Add 10 µl of Heparin (1 mg/ml) into an eppendorf tube labeled as **Inhibitor Control [IC]**. For **Enzyme Control [EC]** and **Background Control [BC]**, add 2 µl of HYAL Assay Buffer into two other eppendorf tubes separately.
- IC₅₀ determination (Optional):** Prepare serial dilutions of the Test Compound (s) using HYAL Assay Buffer. Add 2 µl of diluted compound into the designated tubes.

3. Dilute the stock Human SPAM1 5-fold. For example, for 10 assays, mix 20 μ l of SPAM1 with 80 μ l of HYAL Assay Buffer to prepare the diluted Human SPAM1. Add 10 μ l of diluted Human SPAM1 into [TC], [SC], [IC] and [EC] tubes respectively. Add 10 μ l of HYAL Assay Buffer into the [BC] tube. Adjust the final volume of each tube to 100 μ l using HYAL Assay Buffer.
4. Incubate the tubes at RT for 30 min, protected from light.
5. After 30 min, spin the tubes briefly and transfer 95 μ l of each solution into the desired wells of the pre-coated 96-well plate.
6. Cover the plate with a plate sealer. Incubate the plate at 37 °C for 2 hr.
7. After incubation, aspirate all the samples from each well 3 times. Wash by filling each well with 250 μ l of 1X Wash Buffer and incubating for 30 sec. Remove the 1X Wash Buffer completely before the next wash.
8. Add 50 μ l of HRP Conjugate working solution to the wells.
9. Incubate the plate at RT for 30 min.
10. Aspirate all the reagents and wash buffer from each well 5 times. Wash by filling each well with 250 μ l 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. **Note:** Complete removal of Wash Buffer is essential for accurate results.
11. Add 100 μ l of TMB Substrate to each well. Tap or shake the plate occasionally to ensure complete mixing.
12. Read the OD at 650 nm for [BC]. When the signal at 650 nm reaches approximately 1.0 for the IC, add 50 μ l of Stop Solution to all the wells and gently tap the plate to ensure thorough mixing.
13. Measure the OD of the plate at 450 nm.

VII. Calculation:

Obtain the net absorbance (Δ OD) for all Test Samples [TC], Enzyme Control [EC], Solvent Control [SC], Background Control [BC] and Inhibitor Control [IC] wells by subtracting the reading of the [EC] wells from all readings. If the [SC] reading is significantly different from [EC], then use the corrected [SC] instead of [EC] to determine effect of tested compound. Calculate the % Relative Inhibition as shown below. Plot the Relative Inhibition (%) vs. corresponding compound concentration (μ g/ml or μ M).

$$\text{Relative Inhibition (\%)} = \frac{\Delta\text{OD}[\text{TC}]}{\Delta\text{OD}[\text{BC}]} \times 100$$

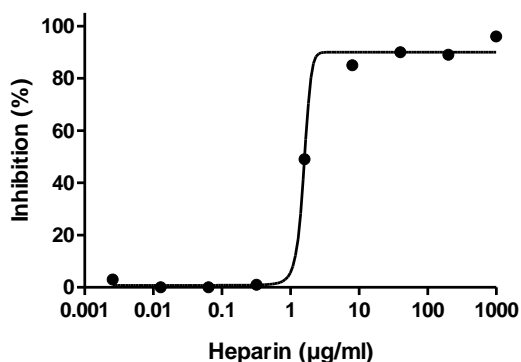


Figure. Inhibition curve of SPAM1 by heparin. IC_{50} of heparin is determined to be ~ 2 μ g/ml using the kit protocol.

IX. Related Products:

Hyaluronidase Activity ELISA Kit (Cat. No. E4909)

Human CellExp™ SPAM1, human recombinant (Cat. No. 7247)

Human CellExp™ Hyaluronidase 1/HYAL1 (Cat. No. P1559)

Biotinylated Hyaluronic Acid (Cat. No. M1325)

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