

HepActiv™ Heparanase Activity Assay Kit

Catalog Number: INS-26-4-0000-10

PURPOSE

HepActiv™ Heparanase Activity Assay Kit allows quick, convenient, high throughput determination of Heparanase enzymatic activity in partially purified or purified Heparanase samples and screening for Heparanase inhibitors using *InSight's* rhHPA1, HepaOne® (INS-26-0-0000-90; INS-26-0-0001-90; INS-26-0-0001-91). This colorimetric kit provides easily interpretable results in 3-4 hours. The kit does not include rHPA1 (HepaOne® may be purchased separately).

BACKGROUND

Heparanase is an endo- β -D-glucuronidase, which degrades heparan sulfate side chains of heparan sulfate proteoglycans (HSPGs) in the extracellular matrix. Heparanase plays an important role in ECM degradation, facilitating the migration and extravasation of tumor cells and inflammatory leukocytes (1,2,3). Upon degradation, Heparanase releases growth factors and cytokines that stimulate cell proliferation and chemotaxis (4,5). Heparanase is a glycosylated heterodimer comprised of a 50 kDa subunit, harboring the active site, and an 8 kDa subunit. It is produced as a latent 65 kDa precursor and proteolytically processed to its active form (1,6).

Heparanase is highly expressed in myeloid leukocytes (i.e. neutrophils) in platelets and in human placenta. Human Heparanase was found to be upregulated in various types of primary tumors, correlating in some cases with increased tumor invasiveness and vascularity and with poor prospective survival (7,8).

PRODUCT

Reagents supplied

1. 96 well filter plate containing immobilized substrate (R plate)
2. Buffer A (reaction mixture) - 10ml
3. Buffer B (sample dilution buffer) - 20ml
4. Buffer C - 10ml
5. Developing reagent - 14ml
6. Plate sealing tape

Equipment required but not supplied

1. 96 well microplate, polystyrene flat bottom (product plate-P plate)
2. Head-over-tail rotator
3. 96 well plate vacuum manifold
4. 37°C incubator
5. 96 well microplate reader capable of measuring absorbance at 530 and 580 nm

METHOD PRINCIPLE

Samples containing Heparanase are incubated with immobilized Heparanase substrate in filter microplates (R plate). Soluble Heparanase-digested molecules released from the immobilized substrate are filtered into a separate receiving microplate (P plate).

Heparanase enzymatic activity is then determined by reacting the collected soluble substrate molecules with a colorimetric reagent. Absorbance at 530 nm is proportional to the amount of released substrate molecules, reflecting Heparanase activity in the sample. For quantitative analysis of Heparanase activity, a standard curve should be included in the assay.

SENSITIVITY

HepActiv™ Heparanase Activity Assay Kit has a detection limit of 0.625 ng of rhHPA1/well and a range of 0.625-80 ng/well.

STORAGE

Upon arrival store buffer C at -20°C. Store other kit components at 2-8°C. Under these conditions **HepActiv™** Heparanase Activity Assay Kit is stable for 12 months.

RESEARCH USE

For *in vitro* research use only. Not for use in diagnostic procedures.

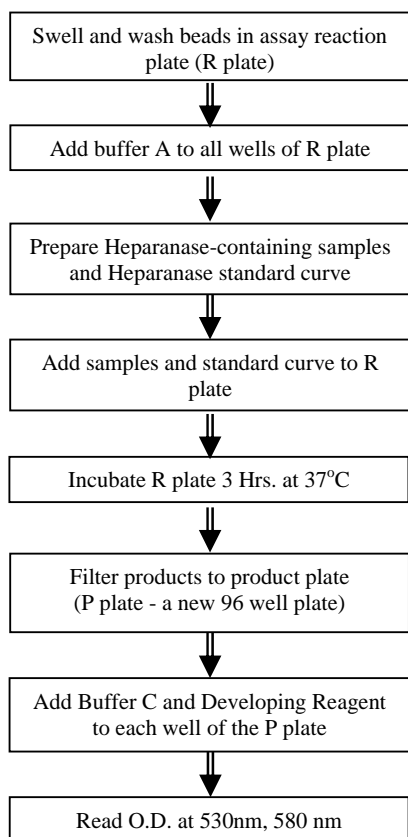
PATENTS

HepActiv™ Heparanase Activity Assay Kit and its constituents are patent protected by US patent no. 6,190,875 and worldwide granted and pending patents and patent applications.

REFERENCES

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ASSAY FLOW DIAGRAM



CRITICAL FACTORS

- All solutions and reagents should be kept on ice until addition to reaction plate.
- Samples should be kept on ice.
- The enzymatic reaction begins immediately upon addition of samples to the reaction plate. Therefore, care should be taken to add samples to the reaction plate simultaneously.
- Prepare the standard Heparanase (rhHPA1) curve on ice right before loading to the reaction plate.
- The assay should be performed using a head-over-tail rotator only. Do not use an orbital shaker.

PROCEDURE

A. Quantitative determination of Heparanase activity

1. Preparation of reaction plate (R plate)

- 1.1. The R plate provided with the assay kit contains beads of immobilized substrate. Before removing the sealing tape, place the plate on the working surface and gently tap the top of the plate to ensure the beads are located at the plate's bottom.
- 1.2. Fill the R plate with distilled water.
- 1.3. Let the beads contained in the plate swell for 1 minute.
- 1.4. Wash the beads twice with distilled water using a vacuum manifold.
- 1.5. Add 80µl buffer A to each well of the R plate.

2. Preparation of samples and Heparanase standard curve

- 2.1. Prepare samples as desired*. If required, dilute samples in cold dilution buffer (buffer B) and keep on ice.
- 2.2. Prepare a standard curve by diluting rhHPA1 standard** in cold buffer B to a concentration of 4000 ng/ml and following 7 serial dilutions of 1:2.

3. Preparation of Heparanase reaction

- 3.1. Transfer 20µl of each standard curve dilution and assayed samples to the R plate. For blank add 20µl of buffer B to two wells. The standard curve range should be 80 to 0.625 ng/well (800-6.25ng/ml). Seal the R plate with the sealing tape and place it onto a **head-over-tail rotator** in a 37°C incubator.
- 3.2. Incubate for 3 hours with constant head-over-tail mixing.

4. Quantification of reaction products

- 4.1. Take the R plate out of the incubator.
- 4.2. Place a new 96 well polystyrene plate (P plate; not provided with assay kit) at the bottom of the vacuum manifold under the gasket holder.
- 4.3. Place the R plate on top of the gasket holder.
- 4.4. Applying the vacuum, filter reaction products to the P plate.
- 4.5. Dispense 70 µl buffer C to each well of the P plate.
- 4.6. Dispense 120 µl of developing reagent to each well of the P plate.
- 4.7. Allow 5 minutes for color development.
- 4.8. Read plate at 530nm and 580nm.
- 4.9. Subtract the O.D at 580nm from the O.D at 530nm.

5. Calculation of results

- 5.1. Plot the Heparanase standard curve on a semi-logarithmic chart. Using 4-parameter logistic fit is recommended.

*To determine background reading it is recommended adding background samples to the reaction (R) plate at the end of the incubation period, before filtering the products to the product plate

** HepaOne® (rhHPA1) is not supplied with the kit and may be purchased separately

B. Screening for Heparanase inhibitors

1. Preparation of reaction plate (R plate)

- 1.1. The R plate provided with the assay kit contains beads of immobilized substrate. Before removing the sealing tape, place the plate on the working surface and gently tap the top of the plate to ensure the beads are located at the plate's bottom.
- 1.2. Fill the R plate with distilled water.
- 1.3. Let the beads contained in the plate swell for 1 minute.
- 1.4. Wash the beads twice with distilled water using a vacuum manifold.
- 1.5. Add 80µl buffer A to each well of the R plate.

2. Addition of compounds to assay plate

- 2.1. Dispense 1µl of each compound to be tested to different wells of the R plate (compounds may be dissolved in DMSO. Final concentration of 1% DMSO does not interfere with the assay).
- 2.2. As a negative control, add 1µl compound diluent to two wells of the R plate.

3. Addition of Heparanase to assay plate

- 3.1. Dilute rhHPA1 in cold buffer B to a concentration of 1000 ng/ml.
- 3.2. Dispense 20µl diluted rhHPA to all well of the R plate except for blank wells. To the blank wells add 20µl of buffer B. The final concentration should be 20 ng/well (200ng/ml).
- 3.3. Seal the reaction plate with the sealing tape and place it onto a **head-over-tail rotator** in a 37°C incubator.
- 3.4. Incubate for 3 hours with constant head-over-tail mixing.

4. Quantification of reaction products

- 4.1. Take the R plate out of the incubator.
- 4.2. Place a new 96 well polystyrene plate (P plate; not provided with assay kit) at the bottom of the vacuum manifold under the gasket holder.
- 4.3. Place the R plate on top of the gasket holder.
- 4.4. Apply vacuum to filter reaction products to the P plate.
- 4.5. Dispense 70 µl buffer C to each well of the P plate.
- 4.6. Dispense 120 µl of developing reagent to each well of the P plate.
- 4.7. Allow 5 minutes for color development.
- 4.8. Read plate at 530nm and 580nm.
- 4.9. Subtract the O.D at 580nm from the O.D at 530nm.
- 4.10. Calculate percent inhibition of each compound from negative control readings.

PERFORMANCE

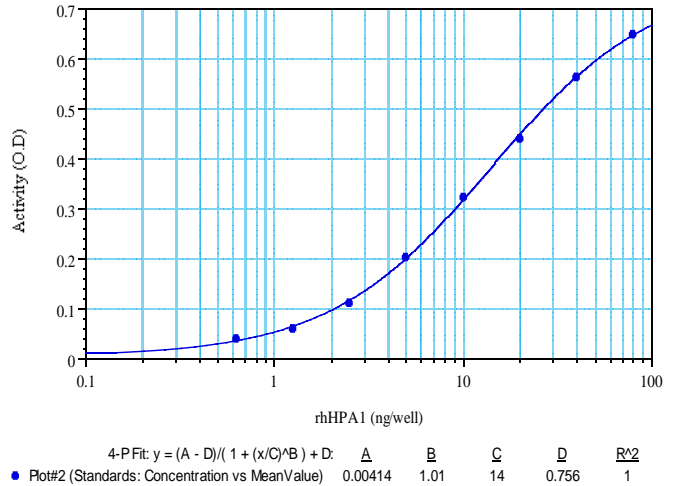


Fig. 1. Activity curve of recombinant human Heparanase standard (rhHPA1) preformed using **HepActiv™** Heparanase Activity Assay kit