

HepAnalyze™ Heparanase ELISA

Catalog Number: INS-26-4-0000-20

PURPOSE

HepAnalyze™ is an enzyme immunoassay (enzyme-linked immunosorbent assay, ELISA) kit for the quantitative measurement of human Heparanase in samples of body fluids, such as plasma and urine and in cell extracts. **HepAnalyze™ is for *in-vitro* research use only and is not intended for human or animal diagnostics or therapeutic uses of any sort.**

BACKGROUND

Heparanase is an endo-β-D-glucuronidase, which degrades heparan sulfate (HS) side chains of heparan sulfate proteoglycans (HSPGs) in the extracellular matrix (ECM). 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 [H65] and proteolytically processed to its active form [H58] (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). Recent data suggest a role for Heparanase in several proteinuric diseases and an increased glomerular Heparanase expression is associated with loss of HS in the glomerular basement membrane (9) leading to albuminuria (10). Heparanase has also been reported to have a role in inflammation and autoimmune diseases (11,12). In addition, independent of its enzymatic activity, Heparanase has roles in cell adhesion and migration and enhancing signaling cascades (13,14).

REFERENCES

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ASSAY PRINCIPLE

The **HepAnalyze™** Heparanase immunoassay is a quantitative solid phase ELISA designed to measure human pro-Heparanase [H65] and the active Heparanase [H58] levels in samples of body fluids and cell extracts. The wells of the microtiter plate are pre-coated with monoclonal antibodies directed against Heparanase. Standard and samples containing Heparanase are incubated in the coated wells and following washing to remove unbound substances, a monoclonal anti-Heparanase antibody conjugated to horseradish peroxidase is added to the wells. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the amount of Heparanase in the samples. Having added the substrate solution, the intensity of the color developed is proportional to the concentration of Heparanase in the samples.

SENSITIVITY

HepAnalyze™ Heparanase ELISA Kit is able to detect pro-Heparanase [H65] and active Heparanase [H58] at a minimum of 55 pg/ml in assayed samples.

RANGE

HepAnalyze™ Heparanase ELISA Kit has a dynamic range of 55-7000 pg/ml.

REAGENTS PROVIDED WITH THE KIT

1. Microtiter plate (96-well), breakable to individual 8-well strips coated with a monoclonal antibody specific to Heparanase
2. Heparanase Standard
3. Assay Diluent. Ready for use
4. Wash Buffer
5. Antibody conjugate: anti-Heparanase monoclonal antibody conjugated to horseradish peroxidase Substrate solution. Ready for use
6. Stop solution. Ready for use
7. Plate sealing tape

EQUIPMENT REQUIRED (NOT SUPPLIED WITH KIT)

1. Manifold dispenser or plate washer
2. Polypropylene tubes or U-bottom 96-well microplate
3. Orbital shaker
4. Pipettes and pipette tips
5. Distilled water
6. Microplate reader for 96-well microplates, capable of measuring absorbance at 450nm

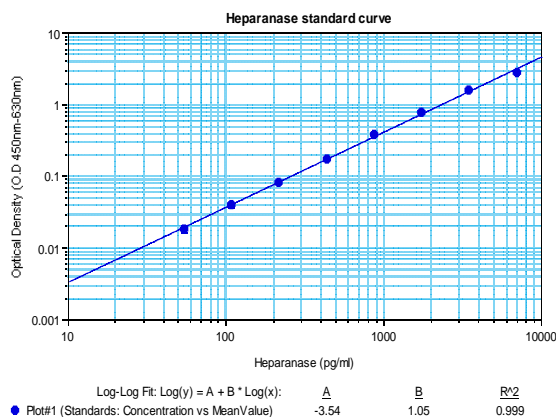
STORAGE

Upon arrival, store Assay Diluent at -20°C. Other kit components should be stored at 2-8°C. Under these conditions **HepAnalyze™** Heparanase ELISA Kit is stable for 3 months.

PERFORMANCE

Standard curve

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



Recovery

Samples have been spiked at known concentrations to human plasma or urine. The % recovery has been calculated.

Sample	Added Heparanase (pg/ml)	Recovery (%)
Plasma	4000	94.4
	2000	94.1
	1000	100.1
	500	101.8
	250	100.7
Urine	4000	93.0
	2000	98.9
	1000	81.4
	500	77.3
	250	93.0

Precision

Intra-assay Precision

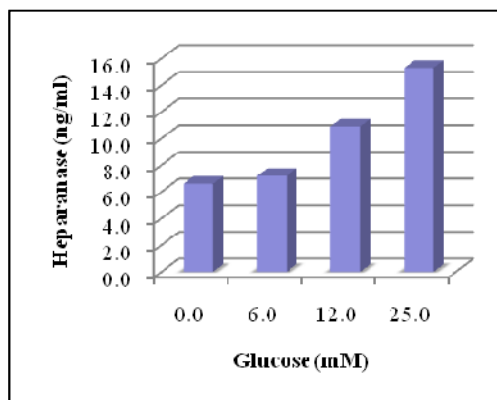
Sample	1	2	3
Mean (pg/ml)	3815.6	2060.6	718.7
Std. Dev. (pg/ml)	196	93.7	44.3
CV (%)	5.1	4.5	6.2
n=	20	20	20

Inter-assay Precision

Sample	1	2	3
Mean (pg/ml)	3848.8	2028.4	724.0
Std. Dev. (pg/ml)	248.8	114.9	29.4
CV (%)	6.46	5.67	4.06
n=	12	12	12

Experimental results

Heparanase gene expression is up-regulated by high glucose leading to decrease in heparan sulfate in the glomerular basement membrane (see Ref. 10).



Determination of glucose-induced Heparanase levels in HEK-293 human embryonic kidney cells. HEK-293 cells were treated with increasing concentrations of glucose. After 48 hrs, equal amounts of protein were subjected to Heparanase quantitation using **HepAnalyze™** Heparanase ELISA kit. Heparanase levels increased in correlation to glucose concentration.

HepAnalyze™ Heparanase ELISA Kit and its constituents are protected by US patents no. 6,177,545; 6,531,129; 7,049,407; 7,666,651; and other worldwide granted and pending patents and patent applications