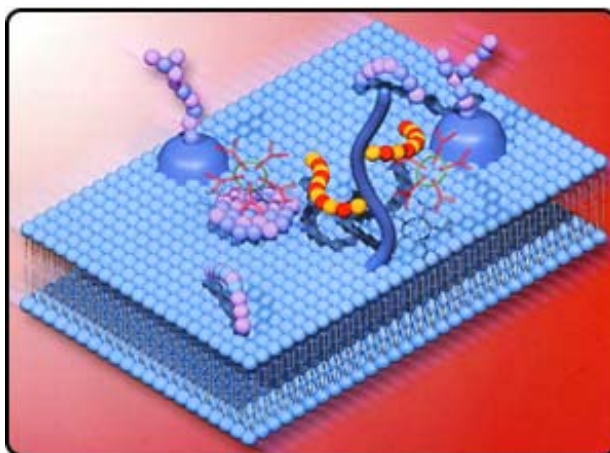


Heparan Sulfate ELISA Kit

(Cat: No. 280564-1)

Technical Information:



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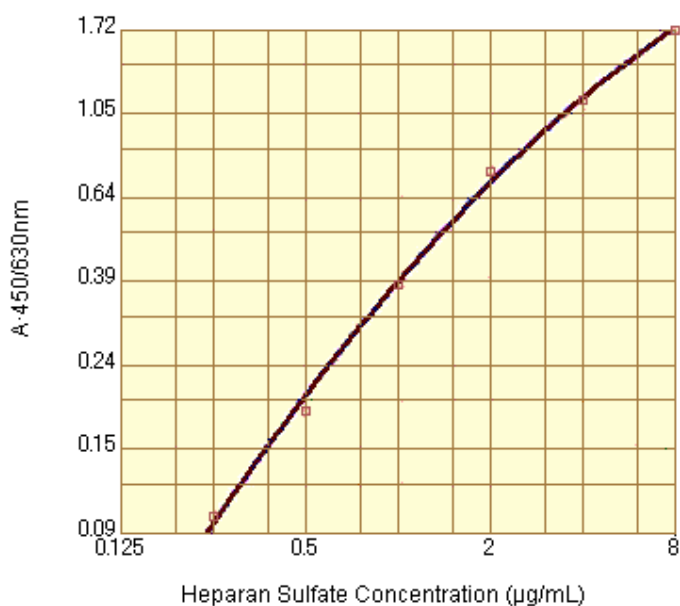
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General Description

Range		0.25-8µg/mL
Measuring time	Primary Reaction	18-24hrs
	Secondary Reaction	60min
	Color Development	30min
Blank OD (0µg/mL)		Less than 0.15
CV (wells, lots, dates, kits)		Less than 20%
Correlation coefficient (r) (log-log quadratic curve)		More than 0.980

◆ Typical Standard Curve



To make the Heparan Sulfate standard curve (quadratic curve), plot the absorbance (blank subtracted) on the x-axis (log), and the Heparan Sulfate concentration on the y-axis (log) using graph paper or appropriate software.

- Basic performance

◆ Accuracy of the kit

The basic performance of the Heparan Sulfate ELISA Kit was evaluated.

▶ Difference between wells in a plate

Three kinds of samples (urine) were measured according to the protocol using four wells per sample (in the same plate).

Sample	HS conc.(µg/mL)				average (µg/mL)	SD	CV(%)
	Well 1	Well 2	Well 3	Well 4			
1	1.94	2.02	2.13	2.25	2.09	0.13	6.2
2	1.56	1.65	1.84	1.88	1.73	0.15	8.7
3	3.37	3.62	4.00	3.85	3.71	0.28	7.5
Average CV (%)							7.5

▶ Difference between kits

Three kinds of samples (urine) were measured according to the protocol using three kits (from the same lot).

Sample	HS conc.(µg/mL)			Average (µg/mL)	SD	CV(%)
	Kit #1	Kit #2	Kit #3			
1	2.09	1.90	2.16	2.05	0.13	6.3
2	1.73	1.64	1.72	1.70	0.05	2.9
3	3.71	3.63	3.83	3.72	0.10	2.7
Average CV (%)						4.0

▶ The difference between measurement dates.

Three kinds of samples (urine) were measured on four different days according to the protocol using the kit (from the same lot).

Sample	HS conc.(µg/mL)				Average (µg/mL)	SD	CV(%)
	Day 1	Day 2	Day 3	Day 4			
1	1.21	1.32	1.43	1.32	1.32	0.09	6.8
2	2.98	3.38	3.10	3.15	3.15	0.17	5.4
3	1.49	1.64	1.72	1.65	1.63	0.10	6.1
Average CV (%)							6.1

▶ The difference between lots.

Three kinds of samples (urine) were measured according to the protocol using the kit (three different lots). Measurement was carried out on the same day.

Sample	HS conc.(µg/mL)			Average (µg/mL)	SD	CV(%)
	Lot.#1	Lot.#2	Lot.#3			
1	1.66	1.46	1.41	1.51	0.13	8.6
2	1.72	1.86	1.63	1.74	0.12	6.9
3	6.51	7.34	6.75	6.87	0.43	6.3
Average CV (%)						7.3

▶ Conclusion

The CV values are less than 10% in each of the above evaluations demonstrating good reproducibility of the kit.

Reactivity - 1

◆ Specificity of Heparan Sulfate: Intersection reactivity with various kinds of GAGs

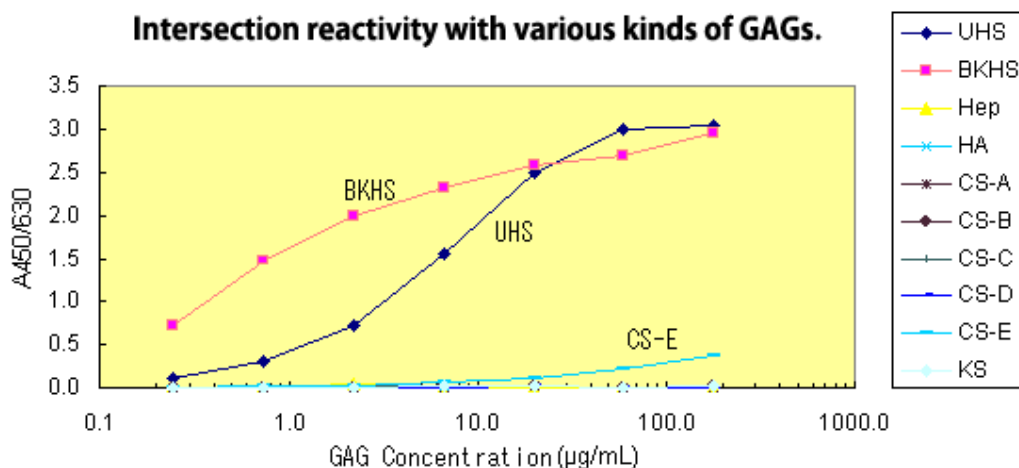
Reactivity of various kinds GAGs was evaluated with the Heparan Sulfate ELISA Kit.

► Method

Three-fold dilution series at 7 concentrations [180.0, 60.0, 20.0, 6.67, 2.22, 0.74 or 0.25 µg/mL] were prepared using the GAGs solution (180µg/mL) shown in the following table and they were measured according to the protocol. OD data are shown in the graph. For Hyaluronic acid, Chondroitin sulfate A, B, C, D, E, and Keratan sulfate, the influence of endogenous Heparan sulfate was minimized by Heparitinase digestion, prior to preparation of the GAG solutions.

[GAGs]				
	GAGs	Origin	Notation	Code#
1	Heparan Sulfate	Human Urine	UHS	-
2	Heparan Sulfate	Bovine Kidney	BKHS	400700
3	Heparin	Pig Intestine	Hep	-
4	Hyaluronic acid	Pig Skin	HA	400720
5	Chondroitin sulfate A	Whale Cartilage	CS-A	400650
6	Chondroitin sulfate B	Pig Skin	CS-B	400660
7	Chondroitin sulfate C	Shark Cartilage	CS-C	400670
8	Chondroitin sulfate D	Shark Cartilage	CS-D	400676
9	Chondroitin sulfate E	Squid Cartilage	CS-E	400678
10	Keratan sulfate	Bovine Cornea	KS	400760

► Results



► Conclusion

The Heparan Sulfate ELISA Kit which reacts with human urine origin Heparan sulfate (UHS) and Bovine kidney origin Heparan sulfate (BKHS) was evaluated with solutions of Heparin, Hyaluronic acid, Chondroitin Sulfate A, B, C, D, and Keratan sulfate in the range 180-0.25µg/mL. Although some reactivity was found in the high concentration solutions (more than 20µg/mL) of Chondroitin sulfate E, it was about 1/100 UHS and about 1/1,000 BKHS and therefore a very weak reaction. Thereby, the Heparan Sulfate ELISA Kit has been shown to be essentially unaffected by other GAGs.

Reactivity - 2

◆ Heparitinase digestive examination

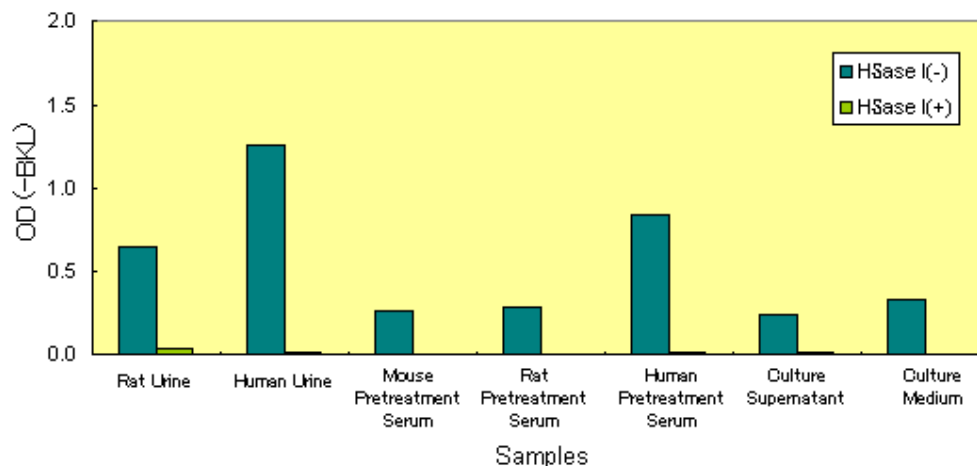
By Heparitinase digestive examination, the specificity of the Heparan sulfate in the various samples was checked with the Heparan Sulfate ELISA Kit

► Method

At room temperature (15-25°C), the samples prepared was measured according to protocol, prior to following heparitinase digestion for 2 hours. For the serum sample, Heparitinase digestion was performed after the serum had been pretreated with Actinase E.

	[GAGs]	
	HSase I (-)	HSase I (+)
Sample	9 vol.	9 vol.
HSase I (1U/mL)	-	1 vol.
Reaction Buffer	1 vol.	-

► Results



* Culture Supernatant: RAW 264.7 (Abelson murine leukemia virus-induced tumor)

** Culture Medium: 10% FBS in alpha- MEM

► Conclusion

All samples had significant reactivity (OD value) prior to heparitinase digestion. However, this disappeared almost completely following the enzyme digestion with Heparitinase I as measured by the Heparan Sulfate ELISA Kit.

Factors affecting measurement-1

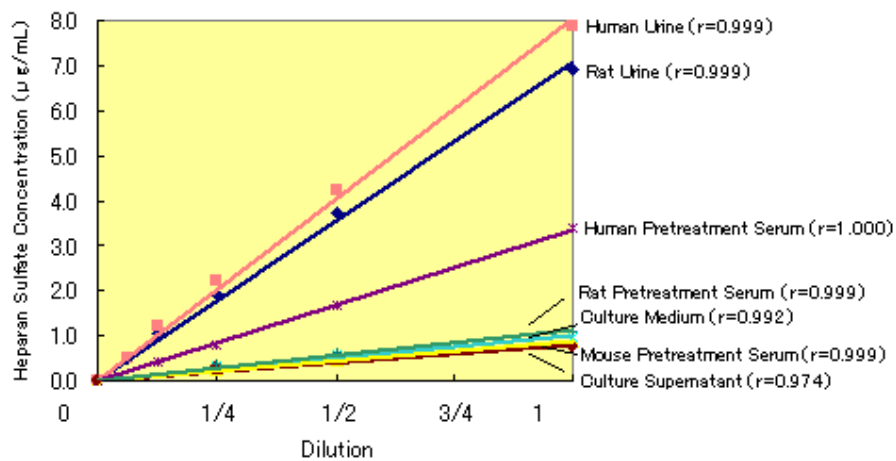
◆ Dilution linearity examination

The dilution linearity of various samples was checked using the Heparan Sulfate ELISA Kit.

► Method

Two double dilution series of each of the samples assayed: 5 concentrations [1 (undiluted solution), 1/2, 1/4, 1/8, 1/16] were prepared and measured according to the protocol. The Heparan sulfate concentrations obtained are shown in the graph and the associated correlation coefficient correlation coefficients (r). For the serum sample, the dilution operation was performed after serum pretreatment with Actinase E

► Results



* Culture Supernatant: RAW 264.7 (Abelson murine leukemia virus-induced tumor)

** Culture Medium: 10% FBS in alpha- MEM

► Conclusion

Good dilution linearity was obtained for each sample with correlation coefficients (r) in the range 0.974-1.000.

Factors affecting measurement - 2

◆ Recovery Test

► Method

A: Addition group	Sample 9 Vol. + HS standard 1 Vol.
B: Non-addition group	Sample 9 Vol. + Buffer 1 Vol.
C: Addition volume	Buffer 9 Vol. + HS standard 1 Vol.

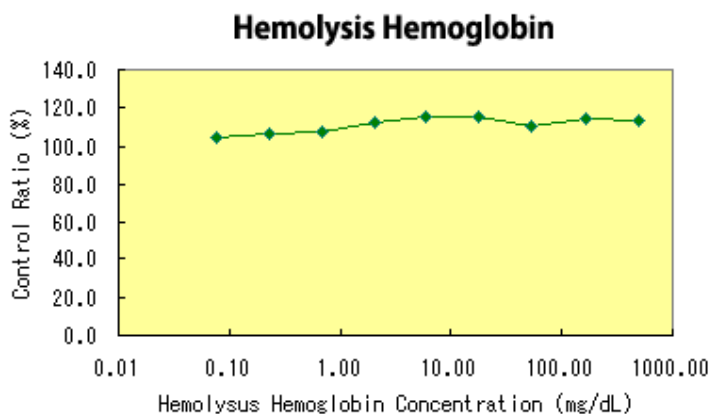
► Result

Sample		Dilution	A: Addition group ($\mu\text{g/mL}$)	B: Non-addition group ($\mu\text{g/mL}$)	Recovery Vol. A-B ($\mu\text{g/mL}$)	C: Addition volume ($\mu\text{g/mL}$)	Recovery (%)
Urine	Rat	x 1/2	5.94	2.93	3.01	3.04	99.0
	Human	x 1	6.74	4.83	1.91	1.94	98.5
Pretreated serum	Mouse	x 1	1.65	0.61	1.04	0.98	106.1
	Rat	x 1/2	2.02	1.24	0.78	0.85	91.8
	Human	x 1	5.10	1.81	3.29	2.80	117.5
Culture media	Cell cultured media	x 1	6.38	3.30	3.08	3.04	101.3
	Medium	x 1	1.80	0.94	0.86	0.90	95.6

Factors affecting measurement - 3

◆ The impact of hemolysis hemoglobin

► Results



Measurement of heparan sulfate in serum

This pretreatment is required in measuring of Heparan Sulfate (HS) in serum from mouse, rat, human and others. This pretreatment is based on digestion of serum proteins by proteinase (actinase E).

"Heparan Sulfate ELISA Kit" does not contain reagents for this pretreatment.

- Method of pretreatment

Instruments and Reagents Required

- Actinase E (KAKEN pharmaceutical CO., LTD. JAPAN)
 - Actinase E Dissolution Buffer (0.5M Tris-HCl, pH7.2-7.5)
 - Water Bath (55°C)
 - Centrifugate
 - Mixer
 - Centrifuge tube or test tube with screw cap (made from polypropylene)^{*1}
- ^{*1}:The tube with screw cap should be airtight avoid to evaporate of sample or inflow water.

Preparation of Reagents

All the reagents prepare before use. Prepared reagents should use promptly and avoid to storage.

[Actinase E Dissolution Buffer (0.5M Tris-HCl buffer, pH7.2-7.5)]

1. Put 6.06g of Tris (hydroxymethyl) aminomethane into 100mL beaker, add 40mL of distilled water (DW), and then dissolve completely.
2. Adjust to pH7.2-7.5 by using 1N HCl.
3. Transfer this solution to 100mL graduated cylinder, fill up to 100mL with DW and then mix well.

[Actinase E Solution]

Dissolve completely actinase E (20mg/mL) in Actinase E Dissolution Buffer.

Procedure for Pretreatment of Serum

1. Add 1 volume of dissolved Actinase E (20mg/mL in actinase E dissolution buffer) against 10 volume of serum & then mix.
2. Digest proteins at 55°C for 16-20hrs. in water bath.
3. After digestion boil for 5min. for stopping digestion.
4. After boil, bring to RT (15-25°C) and then centrifuge 3,000 rpm, for 10mins.
5. After centrifugation, take the supernatant and mix well.
6. The supernatant is applied to Heparan Sulfate ELISA kit.^{*1}

^{*1}:Sample dilution for the measurement with Heparan Sulfate ELISA Kit

Sample	Pretreated Serum		
	Mouse	Rat	Human
Dilution	x 1	x 1/2	x 1

Calculation of Heparan Sulfate Concentration

1. Calculate HS values in pretreated samples according to the Heparan Sulfate ELISA kit procedure.
2. The calculated HS values must be multiplied dilution factors as below to determine the HS concentration in serum.

$$\text{HS concentration} = \text{calculated HS value} \times \text{dilution factor} \times 1.1$$

^{*} As to (diluted) samples that exceeded 8µg/mL, repeat the assay with proper dilution in Reaction Buffer.