

HEPARAN SULFATE ELISA PROTOCOLS

ANTI HEPARAN SULFATE (F58-10E4 & JM-403) ANTI Δ HEPARAN SULFATE (F69-3G10)

Reactivity of 10E4 and 3G10 to BA-HSPG in ELISA

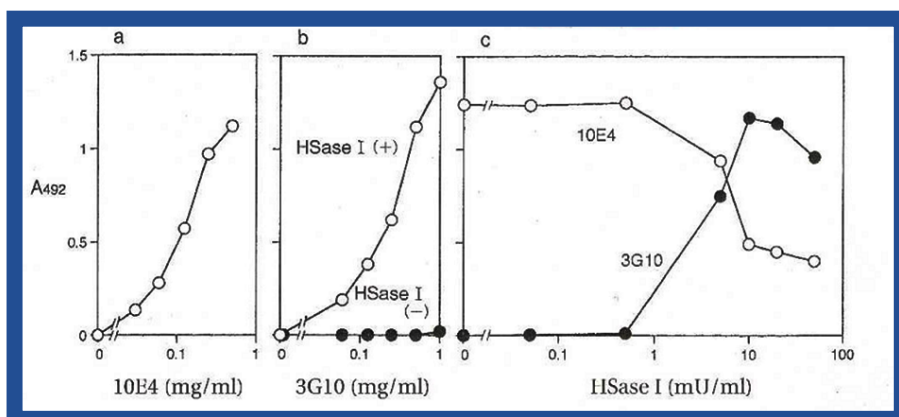
Procedure

1. Coat bovine aorta-derived heparan sulfate proteoglycan (BA-HSPG) to a microplate at 4°C overnight (0.1mg/well)
2. React with Heparitinase I (Heparinase III)* at 37°C for 90 minutes
(optional step: digestion degrades 10E4 epitope but exposes 3G10 epitope - see results below)
3. Wash with 0.5% Tween 20-PBS (-)
4. Block with 3% BSA-PBS (-) at room temperature for 30 minutes
5. Wash with T-PBS
6. React with primary antibody (10E4 or 3G10) at room temperature for 20 minutes
7. Wash with T-PBS
8. React with secondary antibody (peroxidase-labelled rabbit anti-mouse IgG + IgM)†
9. Wash with T-PBS
10. Develop color with o-phenylene diamine /H₂O

* Use Heparitinase I (Seikagaku #. 100704)

† Use HRP-rabbit-anti-mouse IgG + IgM (Jackson # 286255)

Results



a) Reactivity of 10E4: Binding of 10E4 to BA-HSPG was observed in a concentration-dependent manner

b) Reactivity of 3G10: 3G10 was not bound to intact BA-HSPG (●) but bound to BA-HSPG treated with HSase I ((1mU/100ml/well) (○))

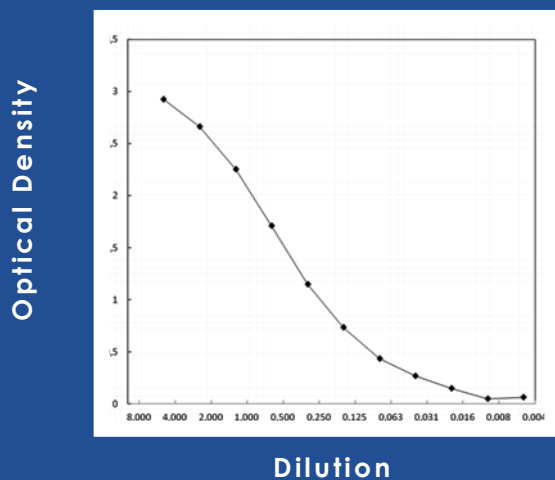
c) Sensitivity of 10E4 and 3G10 to HSase I (antibody concentration 1 mg/ml): Treatment with HSase I decreased the reactivity of 10E4 (●) but increased the reactivity of 3G10 to BA-HSPG

Reactivity of JM403 with heparan sulfate in ELISA

Procedure

1. Coat Heparan sulfate (from Bovine Kidney) 20 µg/ml (in 90% saturated ammonium sulfate) in Maxisorb 96 wells plates (Nunc439454) in 100 µl/well overnight at 4 °C
2. Wash plate 3 times with PBS-0.05% Tween (PBS-T)
3. Block for 1 h at room temperature in 200 µl/well with 1% gelatin (Difco, Bacto 014301) in phosphate buffered saline(PBS)
4. Wash plate 5 times with PBS-T
5. Incubate in 100 µl/well with JM403 serial dilutions Start concentration of JM403 5 ug/ml in PBS for 1 h at room temperature
6. Wash plate 5 times with PBS-T
7. Incubate in 100 µl/well with detecting antibody (secondary antibody) Goat anti mouse-IgM(u-spec)-HRP (Southern Biotechnology, 1021-05) at the appropriate dilution
8. Wash plate 5 times with PBS-T
9. Develop by addition of 100 µl/well TMB solution for 15 minutes at room temperature
10. Stop peroxidase reaction by adding 100 µl/well 2M H₂SO₄
11. Measure absorbance at 450 nm

Results



ORDERING DETAILS

Code	Description	Pack
370255-1	Ab Heparan Sulfate, purified	0.2 mg
370255-S	(clone F58-10E4),	0.05 mg
370260-1	Ab Human D-Heparan Sulfate, purified	0.2 mg
370260-S	(clone F69-3G10),	0.05 mg
370730-1	Ab-Heparan Sulfate (Clone JM-403), Purified	0.2 mg
100704-1A	Heparitinase I (Flavobacterium heparinum)	0.1 unit
AMS.HEP-ENZ III	Heparinase III (Heparitinase I) from Flavobacterium heparinum (EC 4.2.2.8)	0.5 IU
AMS.HEP-ENZ III-S		0.1 IU