

## Monoclonal Anti Heparan Sulfate (JM403 epitope)

### Immunogen:

Heparan Sulfate Proteoglycan from rat glomerular basement membrane

### Specificity:

- Reacts with an epitope present in many types of heparan sulfate.
- Epitope includes (an) N-unsubstituted glucosamine residue(s) that are critical for reactivity.
- Reactivity of the antibody with most heparan sulfates is nearly completely abolished after treatment with bacterial heparitinase (*Flavobacterium heparinum*; EC 4.2.2.8)
- Does not react with heparin, hyaluronan, chondroitin sulfate, dermatan sulfate & keratan sulfate

### Epitope Comparison of JM403 & 10E4 Anti Heparan Sulfate Antibodies

Antibody	Occurrence in HS	Required GlcN N-substitution	Requirement for		Epitope characteristics
			O-Sulfate	IdoUA	
JM403	Common	-NH <sub>3</sub> <sup>+</sup>	No	No	GlcUA-rich sequences with N-unsubstituted GlcN units
10E4	Common	N-Sulfate & N-acetyl	No	No	Mixed N-acetylated /N-Sulfated sequence

### Recommended Applications:

- Immunofluorescence
- ELISA

See protocols and results overleaf

### Ordering Details

Code	Description	Pack
370730-1	Ab-Heparan Sulfate (clone JM-403 epitope), Purified	0.2 mg

AMSBIO | [www.amsbio.com](http://www.amsbio.com) | [info@amsbio.com](mailto:info@amsbio.com)

 **UK & Rest of the World**  
AMS Biotechnology (Europe) Ltd  
184 Park Drive, Milton Park  
Abingdon, UK  
T: +44 (0)1235 828 200  
F: +44 (0) 1235 820 482

 **North America**  
amsbio LLC  
1035 Cambridge Street,  
Cambridge, MA 02141  
T: +1 (617) 945-5033 or  
T: +1 (800) 987-0985  
F: +1 (617) 945-8218

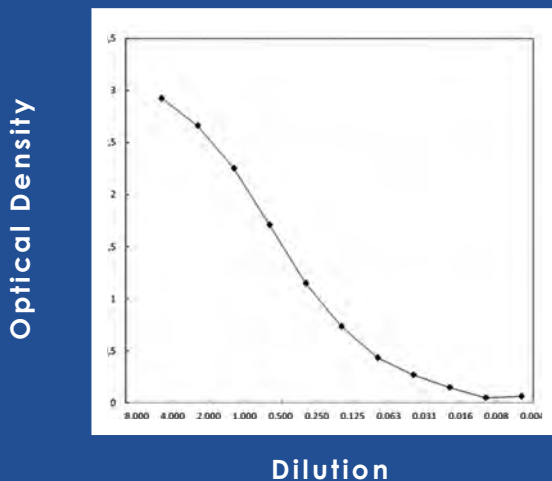
 **Germany**  
AMS Biotechnology (Europe) Ltd  
Bockenheimer Landstr. 17/19  
60325 Frankfurt/Main  
T: +49 (0) 69 779 099  
F: +49 (0) 69 133 76880

 **Switzerland**  
AMS Biotechnology (Europe) Ltd  
Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
T: +41(0) 91 604 55 22  
F: +41(0) 91 605 17 85

## Analysis of reactivity of JM403 with heparan sulfate in ELISA

1. Coat Heparan sulfate(Sigma H7640) 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 H<sub>2</sub>SO<sub>4</sub>
11. Measure absorbance at 450 nm

### JM403: Reactivity with heparin sulphate (bovine kidney) coated in ELISA



AMSBIO | [www.amsbio.com](http://www.amsbio.com) | [info@amsbio.com](mailto:info@amsbio.com)

 **UK & Rest of the World**  
AMS Biotechnology (Europe) Ltd  
184 Park Drive, Milton Park  
Abingdon, UK  
T: +44 (0)1235 828 200  
F: +44 (0) 1235 820 482

 **North America**  
amsbio LLC  
1035 Cambridge Street,  
Cambridge, MA 02141  
T: +1 (617) 945-5033 or  
T: +1 (800) 987-0985  
F: +1 (617) 945-8218

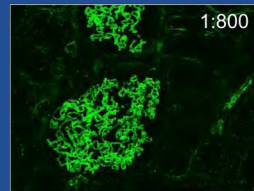
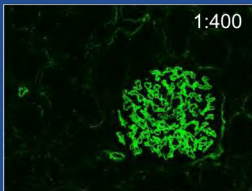
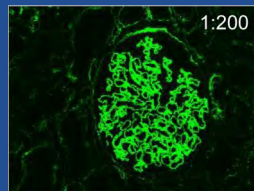
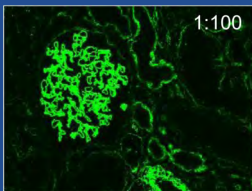
 **Germany**  
AMS Biotechnology (Europe) Ltd  
Bockenheimer Landstr. 17/19  
60325 Frankfurt/Main  
T: +49 (0) 69 779 099  
F: +49 (0) 69 133 76880

 **Switzerland**  
AMS Biotechnology (Europe) Ltd  
Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
T: +41(0) 91 604 55 22  
F: +41(0) 91 605 17 85

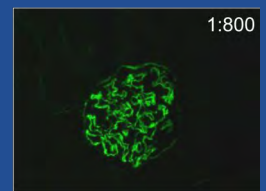
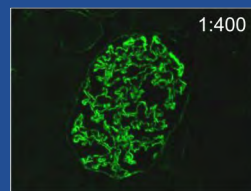
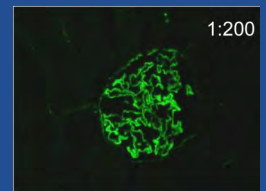
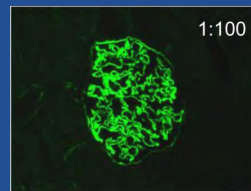
## Immunofluorescence staining of normal frozen kidney (2 μm) sections

- Fix tissue sections for 10 minutes in acetone at 4 °C
- Dry in the air
- Dilute JM403 in PBS containing 1% BSA (1:100 starting dilution for JM403) and incubate for 45 min on sections at room temperature
- Wash three times in PBS
- Dilute detecting antibodies (Goat anti Mouse IgG H&L Alexa488 (Cat #A11001) or Goat anti Mouse IgM Alexa 488 (Cat #A21042) in PBS containing 1% BSA at the appropriate dilution and incubate for 30-45 min at room temperature
- Wash three times in PBS
- Fix with 1% paraformaldehyde in PBS for 15 min
- Wash three times in PBS
- Embed in Vectashield mounting medium

### Immunofluorescence staining on normal human kidney sections (magnification x 20)



### Immunofluorescence staining on normal human kidney sections (magnification x 40)



AMSBIO | [www.amsbio.com](http://www.amsbio.com) | [info@amsbio.com](mailto:info@amsbio.com)

**UK & Rest of the World**  
AMS Biotechnology (Europe) Ltd  
184 Park Drive, Milton Park  
Abingdon, UK  
T: +44 (0)1235 828 200  
F: +44 (0) 1235 820 482

**North America**  
amsbio LLC  
1035 Cambridge Street,  
Cambridge, MA 02141  
T: +1 (617) 945-5033 or  
T: +1 (800) 987-0985  
F: +1 (617) 945-8218

**Germany**  
AMS Biotechnology (Europe) Ltd  
Bockenheimer Landstr. 17/19  
60325 Frankfurt/Main  
T: +49 (0) 69 779 099  
F: +49 (0) 69 133 76880

**Switzerland**  
AMS Biotechnology (Europe) Ltd  
Centro Nord-Sud 2E  
CH-6934 Bioggio-Lugano  
T: +41(0) 91 604 55 22  
F: +41(0) 91 605 17 85