Datasheet for ABIN5509728

Galectin 3 Protein (LGALS3)

1 Validation

Go to Product page

Overview

Quantity:	1 mg
Target:	Galectin 3 (LGALS3)
Origin:	Human
Source:	Escherichia coli (E. coli)
Protein Type:	Recombinant
Biological Activity:	Active
Application:	Functional Studies (Func)

Product Details

Characteristics:	Recombinant Human Galectin-3 (LGALS3)
Purity:	>95 % as determined by SDS-PAGE
Endotoxin Level:	< 1.0 EU of endotoxin/µg of protein

Target Details

Target:	Galectin 3 (LGALS3)
Alternative Name:	Galectin-3 (LGALS3) (LGALS3 Products)
Target Type:	Chemical
Molecular Weight:	26,1
Gene ID:	3958
UniProt:	P17931

Target Details

Pathways:	RTK Signaling

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Reconstitution:	A quick spin of the vial followed by reconstitution in sterile distilled water to a concentration not less than 0.1 mg/mL is recommended. Please note, filter sterilization is a must following reconstitution. This solution can then be diluted into other buffers.
Storage:	4 °C,-20 °C
Storage Comment:	The lyophilized protein is stable for at least one year from date of receipt at -70°C. Upon reconstitution, this cytokine can be stored in working aliquots at 2° - 8°C for one month, or at -20°C for six months, with a carrier protein without detectable loss of activity. Avoid repeated freeze/thaw cycles.

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Successfully validated (Haemagglutination (H))

by Sanja Dabelic, Ph.D., Associate Professor, Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb

Report Number: 103788

Date: Jun 17 2019

Target:	LGALS3
Lot Number:	158844928901
Method validated:	Haemagglutination (H)
Positive Control:	human erythrocytes (blood group A) serum from uncompatible donor (blood group B)
Negative Control:	PBS 0.5mM lactose
Notes:	Passed. The recombinant human galectin-3 ABIN5509728 possesses lectin activity.
Protocol:	• Isolate human erythrocytes and prepare trypsinized,glutaraldehyde-fixed erythrocytes ◦ Take blood by venipuncture from healthy volunteer (blood type A) in one lavender vacutainer tube (containing EDTA as anticoagulant) and centrifuged at 500×g for 5min at 4°C. ◦ Remove plasma and white cell ghost layer at the top of the pellet. ◦ Resuspend red blood cells in 10ml cold PBS by gently aspirating and expelling them with a pipette. Centrifuge red blood cells at 500×g for 5min at 4°C. Remove the supernatant and repeat 3x. ◦ Resuspend the pellet in 20ml PBS (Gibco) containing 0.05% trypsin and incubate at 37°C for 1h with an occasional shake. ◦ Centrifuge at 500xg for 5min at 4°C. ◦ Remove supernatant and resuspend erythrocytes in 10ml cold PBS. ◦ Wash pellet 3x as described above. ◦ Resuspend erythrocytes in 25ml PBS containing 1% glutaraldehyde and incubate for 1h at RT with continuous gentle shaking. ◦ Centrifuge at 500xg for 5min at 4°C. ◦ Remove supernatant and wash erythrocytes 2x with 0.1M glycine in PBS. ◦ Wash erythrocytes with PBS. ◦ Store erythrocytes as 4% (v/v) erythrocyte suspension at 4 °C until use (within one month of preparation). • Isolation of human serum (blood type B) ◦ Take blood by venipuncture from healthy volunteer (blood type B) in one red topped vacutainer tube (additive-free vacutainer).

- Allow the blood to clot by leaving it undisturbed at room temperature for 30min.
- Centrifuge at 1000xg for 10min at 4°C.
- Remove serum supernatant and store at -20°C until use.

- Preparation of galectin-3 and lactose
  - Reconstitute lyophilized galectin-3 (antibodies-online, ABIN5509728, lot 0158844928901) in sterile, 0.2µm filtered solution of 0.2% bovine serum albumin (BSA) in PBS, according to the manufacturers' recommendations, to 100µg/ml.
  - Dilute D-lactose in PBS to 2mM.
- Measurement of Hemagglutination
  - Prepare samples in triplicates as indicated in Figure A in a total volume of 100µl in a microtiter V plate.
  - Incubate plate for 1h and 24h at 4°C.
  - Determine results by visual examination. Image acquisition on an Amersham Imager 600, Image capturing method colorimetric-transillumination.
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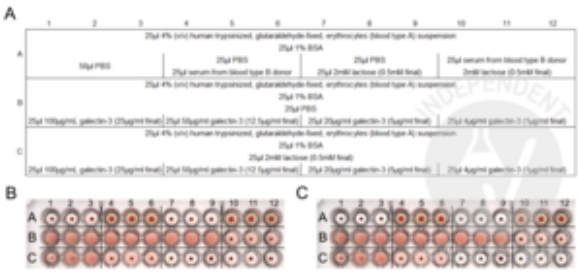
Experimental Notes:

- Galectin-3 is approximately 30kDa and, like all galectins, contains a carbohydrate-recognition-binding domain (CRD) of about 130 amino acids that enable the specific binding of β-galactosides. For several decades, hemagglutination assay has been used to obtain semi-quantitative data on the sugar binding and specificity of a particular lectin. Active lectins agglutinate erythrocytes by recognizing carbohydrates on the cell surface and forming a cross-linked network in suspension.
- By serially diluting the lectin in a 96-well microtiter plate and adding a constant quantity of erythrocytes, the lectin activity can be estimated. Galectin-3 lectin activity is abolished by the addition of lactose, which binds to its CRD domain and consequently inhibits hemagglutination. The assay is usually performed on trypsinized, glutaraldehyde-fixed red blood cells because they are more sensitive to hemagglutination reactions. Addition of bovine serum albumin (BSA) to all wells prevents non-specific interactions and consequently non-specific auto-hemagglutination. As positive control, serum of a non-compatible blood type can be used. Negative control is PBS. Control for abolishing galectin-3 activity is lactose.
- The hemagglutination assay is carried out by incubating human erythrocytes (blood group A or B) in the presence of increasing concentrations of galectin-3 in a microtiter V plate for 1h and 24h at RT in a final volume of 100µl. Galectin-3 recognizes glycans on both type A and type B red blood cells at submicromolar concentrations, according to the literature data, but does not bind to blood group O(H) at these concentrations. Commercially available 4% solutions of trypsinized, glutaraldehyde-fixed erythrocytes from various species and (e.g. rabbit) can also been used.
- Pre-incubation of galectin-3 for 30min in the presence of 500µM lactose should cause prevention (inhibition) of hemagglutination.
- Hemagglutination was detected in wells that contained the positive control without (wells A4-A6) and with lactose (wells A10-12), and in wells with galectin-3 at final concentrations 25µg/mL, 12.5µg/mL, and 5µg/mL (wells B1-9). A galectin-3 concentration of 1µg/ml was insufficient to cause complete hemagglutination (wells B10 to B12). Partial inhibition was detected in wells containing galectin-3 at 25µg/ml in the presence of 0.5mM lactose (well C1-

3). At galectin-3 concentrations 12.5µg/ml, 5µg/ml, and 1µg/ml hemagglutination is completely abolished in the presence of 0.5mM lactose (well C4-12).

- References:
- https://www.ncbi.nlm.nih.gov/pubmed/1259723
- https://www.ncbi.nlm.nih.gov/pubmed/10722666
- https://www.ncbi.nlm.nih.gov/pubmed/22497898
- https://www.ncbi.nlm.nih.gov/pubmed/27328612
- https://www.ncbi.nlm.nih.gov/pubmed/30797745
- https://www.ncbi.nlm.nih.gov/pubmed/18216021

Image for Validation report #103788



Validation image no. 1 for Galectin 3 (LGALS3) (Active) protein (ABIN5509728)

Hemagglutination assay using recombinant human galectin-3 ABIN5509728. A. Plate Layout. B. and C. Pictures captured after 1h and 24h respectively.