Galectin 1 Monoclonal Antibody (6C8.4-1)

**Catalog Number** 43-7400

<table>
<thead>
<tr>
<th>Details</th>
<th>Species Reactivity</th>
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<tbody>
<tr>
<td>Size</td>
<td>100 µg</td>
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<tr>
<td>Host/Isotope</td>
<td>Mouse / IgG1</td>
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<tr>
<td>Class</td>
<td>Monoclonal</td>
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<tr>
<td>Type</td>
<td>Antibody</td>
</tr>
<tr>
<td>Clone</td>
<td>6C8.4-1</td>
</tr>
<tr>
<td>Immunogen</td>
<td>Mixture of two peptides from the middle and C-terminal regions of human Galectin-1 protein</td>
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<tr>
<td>Conjugate</td>
<td>Unconjugated</td>
</tr>
<tr>
<td>Form</td>
<td>Liquid</td>
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<tr>
<td>Concentration</td>
<td>0.5 mg/mL</td>
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<tr>
<td>Purification</td>
<td>Protein A</td>
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<tr>
<td>Storage buffer</td>
<td>PBS, pH 7.4</td>
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<tr>
<td>Contains</td>
<td>0.1% sodium azide</td>
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<tr>
<td>Storage Conditions</td>
<td>Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.</td>
</tr>
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**Specified Information**

The peptides used for immunization have ~90% similarity to swine, rat, ovine, Chinese hamster sequences and 80% similar to bovine and mouse sequences.

**Background/Target Information**

Galectin-1 belongs to a large family of carbohydrate-binding proteins called lectins. Galectin-1 can be either monomeric or homodimeric and is found in a wide variety of cells and tissue types. Galectin-1 can control cell growth, proliferation, induce apoptosis of activated T cells while it can also modulate cytokine secretion or inhibit pro-inflammatory cytokine production. Galectin-1 plays an important role in acute and chronic inflammation.


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Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. PC-3 cells were transfected with Galectin 1 siRNA and decrease in signal intensity was observed in Western Blot application using Anti-Galectin 1 Monoclonal Antibody (6C8.4-1) (Product # 43-7400). 

Knockdown validation info.

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Immunofluorescence analysis using Anti-Galectin 1 Monoclonal Antibody (6C8.4-1) (Product # 43-7400), shows cytoplasmic and membrane staining for Galectin 1 in MDA-MB-231 cells, a triple negative breast cancer cell line, in comparison to non-TNBC cell line MCF7. Relative expression validation info.

### Advanced Verification Data

**Galectin 1 Antibody (43-7400)**

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- Knockdown validation info.

**Galectin 1 Antibody (43-7400)**

- Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Immunofluorescence analysis using Anti-Galectin 1 Monoclonal Antibody (6C8.4-1) (Product # 43-7400), shows cytoplasmic and membrane staining for Galectin 1 in MDA-MB-231 cells, a triple negative breast cancer cell line, in comparison to non-TNBC cell line MCF7. Relative expression validation info.

### Product Images For Galectin 1 Monoclonal Antibody (6C8.4-1)

**Galectin 1 Antibody (43-7400) in ICC/IF**

Immunofluorescence analysis of Galectin 1 was performed using 70% confluent log phase MDA-MB-231 and MCF7 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™-X-100 for 10 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with Galectin 1 Monoclonal Antibody (6C8.4-1) (Product # 43-7400) at 1:100 in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32766), (1:2500 dilution), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b: Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasmic and membrane localization of Galectin 1 in MDA-MB-231. Panel e represents MCF7 cells showing negative staining for the same. Panel f represents control MDA-MB-231 cells with no primary antibody to assess background. The images were captured at 60X magnification.


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Galecint 1 Antibody (43-7400) in ICC/IF

Immunofluorescence analysis of Galecint 1 was done on 70% confluent log phase U-87MG cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Galecint 1 (6C8.4-1) Mouse Monoclonal Antibody (Product # 43-7400) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 555 Rhodamine Phalloidin (Product # R415, 1:300). Panel d is a merged image showing cytoplasmic localization. Panel e is a no primary antibody control. The images were captured at 60X magnification.

Galecint 1 Antibody (43-7400) in IHC (P)

Immunohistochemistry analysis of Galecint 1 showing staining in the cytoplasm and nucleus of paraffin-embedded human esophage tissue (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Anti-Galecint 1 Monoclonal Antibody (Product # 43-7400) diluted in 3% BSA-PBS at a dilution of 1:20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

Galecint 1 Antibody (43-7400) in IHC (P)

Immunohistochemistry analysis of Galecint 1 showing staining in the cytoplasm and nucleus of paraffin-embedded human kidney tissue (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Anti-Galecint 1 Monoclonal Antibody (Product # 43-7400) diluted in 3% BSA-PBS at a dilution of 1:100 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

Galecint 1 Antibody (43-7400) in IHC (P)

Immunohistochemistry analysis of Galecint 1 showing staining in the cytoplasm and nucleus of paraffin-embedded mouse ovary tissue (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Anti-Galecint 1 Monoclonal Antibody (Product # 43-7400) diluted in 3% BSA-PBS at a dilution of 1:50 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.


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Galectin 1 Antibody (43-7400) in WB
Western blot was performed using Anti-Galectin 1 Monoclonal Antibody (6C8-4-1) (Product # 43-7400) and a 15kDa band corresponding to Galectin 1 was observed across PC-3, THP-1, MDA-MB-231 but not in LNCaP and MCF7 and in Mouse Adipose. Whole cell extracts (30 µg lysate) of PC-3 (Lane 1), LNCaP (Lane 2), THP-1 (Lane 3), MDA-MB-231 (Lane 4), MCF7 (Lane 5) and tissue extracts (30 ug lysate) of Mouse Adipose (Lane 6) were electrophoresed using NuPAGE™ 12% Bis-Tris Protein Gel (Product # NP0341BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Produced # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A28177,1:8000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). Increased expression of Galectin-1 is seen in triple negative breast cancer (TNBC) (like MDA-MB-231) for promoting metastasis but not in non-TNBC like MCF7 [10.18632/oncotarget.16208] and in androgen unresponsive PC-3 cells in comparison to androgen responsive LNCaP [10.1155/2013/519436]. The band at ~25 kDa in Mouse Adipose corresponds to circulating IgGs that are commonly detected in mouse tissue lysates.

Galectin 1 Antibody (43-7400) in WB
Western blot analysis was performed on whole cell extracts (30 µg lysate) of THP-1. The blots were probed with Anti-Galectin 1 Mouse Monoclonal Antibody (Product # 43-7400, 1-2 µg/mL) and detected by chemiluminescence Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP conjugate (Product # 62-6520, 1:4000 dilution). A 14 kDa band corresponding to Galectin 1 was observed across cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 12 % Bis-Tris gel (Product # NP0342BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane using iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).

Galectin 1 Antibody (43-7400) in WB
Knockdown of Galectin 1 was achieved by transfecting PC-3 with Galectin 1 specific siRNAs (Silencer® select Product # s194592, s8145). Western blot analysis (Fig. a) was performed using whole cell extracts (40 ug lysate) from the Galectin 1 knockdown cells (Lane 3), non-targeting scrambled siRNA transfected cells (Lane 2) and untransfected cells (Lane 1). The blot was probed with Galectin 1 Monoclonal Antibody (6C8-4-1) (Product # 43-7400, 1:1000 dilution ) and Goat anti-Mouse IgG (H+L) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A28177, 1:8000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to Galectin 1.


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PubMed References For Galectin 1 Monoclonal Antibody (6C8.4-1)

1 Western Blot References

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<th>Summary</th>
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<tr>
<td>Pig / 1:500</td>
<td>43-7400 was used in Western Blotting to propose the Mascot-jTRAQx-CiR-C strategy as a simple yet powerful data processing adjunct to the iTRAQ technology.</td>
</tr>
</tbody>
</table>

Bioscience reports (Jun 2019; 39: null)
"Sum of peak intensities outperforms peak area integration in iTRAQ protein expression measurement by LC-MS/MS using a TripleTOF 5600+ platform."
Author(s): Burat B, Gonzalez J, Sauvage F.L., Amouad H, Arion H, Pinault E, Marquet P, Essig M
PubMed Article URL: http://dx.doi.org/10.1042/BSR20190904

1 Flow Cytometry References

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<td>Human / Not Cited</td>
<td>43-7400 was used in flow cytometry to examine the role of human iNKT cells in HSV-1 infection and study their interaction with epidermal keratinocytes</td>
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Journal of immunology (Baltimore, Md. : 1950) (Jun 2012; 188: 6216)
"Contact-dependent interference with invariant NKT cell activation by herpes simplex virus-infected cells."
Author(s): Bosnjak L, Sahlström P, Paquin-Proulx D, Leamensyah E, Moll M, Sandberg JK
PubMed Article URL: http://dx.doi.org/10.4049/jimmunol.1100218