Sheep antibody to Connexin-40 (254-270)/Cx40/254 antisera: whole serum

Catalogue No.:	S-063-100
Description:	FUNCTION: One gap junction consists of a cluster of closely packed pairs of transmembrane channels, the connexons, through which materials of low molecular weight diffuse from one cell to a neighboring cell. SUBUNIT: A connexon is composed of a hexamer of connexins. SUBCELLULAR LOCATION: Membrane; multi-pass membrane protein. TISSUE SPECIFICITY: Highly expressed in lung. SIMILARITY: Belongs to the connexin family. Alpha-type (group II) subfamily.
Batch No.:	See product label
Unit size:	100 uL
Antigen:	A synthetic peptide consisting of amino acids 254 to 270 of rat Cx40 (Cx40/254) conjugated to diphtheria toxoid has been used as the immunogen.
Sequence:	SLVQGLTPPPDFNQCLK
Antibody Type:	Polyclonal
Other Names:	Gap junction alpha-5 protein; Cx40; Gja5; Cxn-40
Accession:	P28234 CXA5_RAT
Produced in:	Sheep
Molecular Weight:	40kDa, Cx40/254 antisera is not recommend for western blots
Purity:	Whole serum
Applications:	Immunohistochemistry: Antibody detects Cxn 40 in rat tissues and arterial endothelial cells. The authors report that the density of Cx40 plaques was significantly greater in the caudal artery (CA) than in the thoracic aorta (ThA), whereas no such difference was seen for Cx37 and Cx43. Expression of Cx40 was absent from the media of both thoracic and caudal artery tissues (see Rummery, NM et al 2002 for more staining specifics). Published Method: Unfixed 10 ?m thick sections cryosections or lightly fixed (2% paraformaldehyde in 0.1 mol/L sodium phosphate buffer) whole mount sections have been tested, see Rummery, NM et al 2002). Pretreatments include pre-incubation for 30 minutes in a blocking solution of 2% bovine serum albumin (BSA), 0.2% Triton-X in PBS, followed by primary antibody incubation. Antibody was used at 1:100 to 1:250 for 1 hour in the original work but Biosensis recommends optimizing the conditions for the best results. Original detection was via Cy3- conjugated anti-goat immunoglobulins (Jackson Immunoresearch Laboratories Inc, PA, 1:100) in 0.01% Triton-X in PBS, but other secondary conditions should work as well once optimized. In the original work the specificity of each antibody was tested by incubation either without primary antibody or with primary antibody that had previously been pre-incubated for 1 hour at room temperature with 10-fold excess by weight of the peptide against which the antibody was raised. (Adapted from Rummery, NM et al 2002).Western Blot: Antibody is not recommended for western blots by Biosensis, however, it does react in westerns with Cxn 40 specific material. The authors report that the antibody develops numerous bands in westerns blots, only some of which are removed

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upon peptide treatment (see Rummery, NM et al 2002). The Cx40/254 antibody specifically recognized a band of 40 kDa from lung, caudal artery (CA), and thoracic aorta (ThA) but not liver (online Figure VIIA, +/- peptide). In the lung, however, a band at 45 kDa also appeared to be reduced with Cxn 40 peptide addition.Published Method: Brain, heart, liver, lung, thoracic aorta and caudal arteries were removed from 5-6 week old Wistar rats and snap frozen in liquid nitrogen Tissues were ground under liquid nitrogen in a mortar and pestle and resuspended in 1 mL of lysis buffer (1 mM NaHCO3 pH 7.05, 10 mM EDTA, 10 mM Iodoacetamide, 10 mM tetra-sodium pyrophosphate, 1 mM PMSF and 1 ug/mL each of antipain, aprotinin, pepstatin-A, chymostatin and leupeptin). Tissues were further disrupted by grinding in a polytron blender. Unbroken cells and large debris were removed by centrifugation at 1000 g for 5 minutes at 4oC, the supernatant was then removed and centrifuged at 3000 g for 5 minutes. The pellet was discarded, and the supernatant centrifuged at 20000 g for 15 minutes at 4?C. The supernatant was discarded, and the membrane-enriched pellet was resuspended in lysis buffer. Protein concentration was measured using the Bio-Rad protein assay kit. Membrane-bound connexins were subsequently solubilized by incubation in 2x SDS sample buffer (5% SDS, 125 mM Tris-Cl (pH 6.8), 20% glycerol, 2 mM -beta- mercaptoethanol, 0.1% (w/v) bromophenol blue) for 60 minutes at 37?C. Aliquots containing 5 ug of protein were separated by SDS-PAGE on 12% polyacrylamide gels and blotted onto PVDF membranes. Blots were probed with sheep antibodies against Cx40 (1:1000, Cx40/254) and detected via ECL using anti-Goat poly HRP secondary antibodies 1:4000, 1 hr. (adapted from Rummery, NM et al 2002).Biosensis recommends optimal dilutions/concentrations should be determined by the end user.

Species Against: This antiserum recognizes Connexin-40 in rat, other species not yet tested

Cross-reactivity: This antibody may show some reactivity with Cxn 45 in Western Blotting only.

Form: Lyophilised

Reconstitution: Reconstitute in 100 uL of sterile water. Centrifuge to remove any insoluble material.

Storage: Store lyophilized antibody at 2-8C. After reconstitution keep aliquots at -20C for a higher stability, and at 2-8C with an appropriate antibacterial agent. Glycerol (1:1) may be added for an additional stability. Avoid repetitive freeze/thaw cycles.

Expiry Date: 12 months after purchase

Specific References: Original Reference

Rummery NM, Hickey H, McGurk G, Hill CE. (2002) “Connexin37 is the major connexin expressed in the media of caudal artery.” Arterioscler Thromb Vasc Biol. 22(9):1427-32. PMID: 12231561This antibody is referred to as Cx40/254 in Rummery, NM et al 2002.

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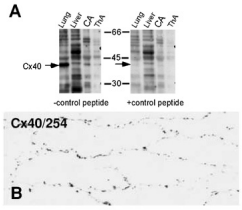

A: Western blotting of tissue extracts from rat brain, heart, lung, liver, caudal artery (CA) and thoracic aorta (ThA) using S-063-100. Arrows show the position of the expected Cx band. The left panels represent incubation with Cx antibody whereas right panels represent pre-incubation of the antibody with immunogenic peptide (adapted from Rummery, NM et al 2002, Supplementary Figure VII.)

B: Immunohistochemistry. En face view of Cx expression in endothelial cells of rat thoracic aorta with antibody S-063-100. Plaques can be seen outlining the perimeter of endothelial cells (adapted from Rummery, NM et al. 2002, Supplementary Figure IV.)

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