# Affinity Purified Rabbit Anti-Phospho-Connexin43 (S368) Certificate of Analysis

#### ORDERING INFORMATION

Catalog Number: PPS046

Lot Numbers: 1658672

Size: 100 µL (sufficient for 10 mini-blots)

Storage: ≤ -20° C

Specificity: Human, mouse, rat, and non-

human primate ~43 kDa Connexin43 phosphorylated at

S368

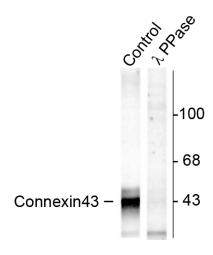
Immunogen: Phosphopeptide corresponding

to amino acid residues surrounding the phospho-S368

of rat Connexin43

Ig Type: rabbit IgG

Applications: Western blot



Western blot of rat hippocampal lysate showing specific immunolabeling of the approximately 43 kDa Connexin43 phosphorylated at S368 (Control). The phosphospecificity of this labeling is demonstrated by treatment with 1200 units of lambda phosphatase ( $\lambda$  PPase) for 30 minutes before being exposed to the Anti-Phospho-Connexin43 (S368). The immunolabeling of Connexin43 is completely eliminated by treatment with  $\lambda$  PPase.

#### Description

Connexin43 (Cx43; also gap junction  $\alpha$ -1 protein) is a 41 - 44 kDa member of the connexin family,  $\alpha$ -type subfamily, of transmembrane proteins. It is the most common type of connexin in cardiac muscle cells, and also occurs in hepatocytes, astrocytes and ovary granulosa cells. It is a 4-transmembrane protein 382 amino acids (aa) in length that contains two cytoplasmic tails. One is 12 aa in length at the N-terminus and the second is 151 aa in length at the C-terminus. Human and rat Cx43 are 98% as identical over the entire length of the molecule. Connexins form gap junctions (GJs) which are intercellular channels between cells. Each adjacent cell contributes to a functional channel. The fundamental unit is a "connexon", or hemi-channel, which is composed of six connexins in a sliding subunit hexamer configuration. The hexamer may be either homomeric or heteromeric. The connexon arrangement provides for the opening and closing of an intersubunit space (or pore) that allows diffusion of molecules 1 kDa or less. It is suggested that the extended C-terminus of each connexin may interact with multiple docking proteins and serve as a plug during closure. Connexons on adjacent cells interact via their extracellular loops to form a GJ channel. When open, these channels allow for the transit of small hydrophilic molecules such as ATP, glucose, IP3 and Ca<sup>++</sup>. Connexons (or hemi-channels) that do not interact with adjacent cells are also suggested to allow for small molecule transit under unusual circumstances. Cx43 activity is regulated by phosphorylation. The exact effects, however, may be context-specific. In one case, PKC phosphorylation of S368 has been found to decrease GJ channel permeability to select small molecular weight solutes. Alternatively, PKA has been suggested to be the mediator of Cx43 phosphorylation of S365, S368, S369 and S373. In this case, phosphorylation promotes channel activity.

## Preparation

Prepared from rabbit serum by affinity purification via sequential chromatography on phospho and dephosphopeptide coupled columns.

#### **Formulation**

100  $\mu$ L in 10 mM HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/mL BSA and 50% glycerol.

#### Storage

For long-term storage,  $\leq$  -20° C is recommended. Product is stable at  $\leq$  -20° C for at least 1 year.

### **Specificity**

This antibody is specific for the 43 kDa Connexin43 in Western blots of rat brain lysates.

# Applications

Western blot - 1:1000

Optimal dilutions should be determined by each laboratory for each application.

#### References

- 1. Sohl, G. and K. Willecke (2004) Cardiovasc. Res. 62:228.
- 2. Willecke, K. et al. (2002) Biol. Chem. 383:725.
- 3. Giepmans, B.N.G. (2004) Cardiovasc. Res. **62**:233.
- 4. Vinken, M. et al. (2006) Cell. Signal. 18:592.
- 5. Stout, C. et al. (2004) Curr. Opin. Cell Biol. 16:507.
- 6. Bao, X. et al. (2004) Am. J. Physiol. Cell. Physiol. 286:C647.
- 7. Yogo, K. et al. (2006) J. Reprod. Dev. **52**:321.

Helen Zhang

Quality & Regulatory Affairs

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc. 1-800-343-7475

716182.0 10/06