Mouse/Rat Glucose Transporter (Glut-1) antibodies and Positive Controls

Cat. # GT11-S , Rabbit Anti-Rat Glut-1 Antibody # 1, 100 µl neat serum,

Cat. # GT11-A , Rabbit Anti-Rat Glut-1 Antibody # 1(affinity pure), 100 µg,

Cat. # GT11-P, Rat GT11 Control peptide # 1, 100 µg/100 µl ,

Cat. # GT11-C, Rat Glut-1 Positive Control for Western Blot, 100 µl

Source of Antigen, Antibodies, and Positive Controls

A 12 AA peptide sequence (designated as GT11-P, control peptide) near the C-terminus of mouse/rat brain glucose transporter-1 (Glut-1) was selected for antibody production (1). A cysteine has been added at the NH2 terminus for coupling to KLH. The peptide was coupled to KLH and antibodies generated in rabbits. Antibody has been affinity purified using control peptide-Sepharose. Rat Glut-1 positive control was prepared from purified rat brain membranes and supplied in SDS-PAGE sample buffer.

Form & Storage

Control peptide Solution is provided in PBS, pH 7.4 at 1 mg/ml (100 µg/100 µl). Antiserum is supplied as neat serum (100 µl soln or lyophilized). Affinity pure antibodies were purified over the peptide-Sepharose column and supplied as 1 mg/ml soln in PBS, pH 7.4 and 0.1% BSA as stabilizer (100 µl in solution or Lyophilized). The peptides and antibodies also contain 0.02% sodium merthiolate as preservative.

Positive Control for Western blot is supplied in SDS-PAGE sample buffer (Laemmeli buffer, reduced). The solution is supplied ready-to-use and no heating is required before use. It should be stored frozen in small aliquots. It is not necessary to reheat the solution before use. Simply thaw it or bring it to room temperature to dissolve any precipitated SDS. Do not freeze and thaw or heat because it may cause irreversible aggregation of proteins and generation of additional high mol. wt. bands.

It can be directly applied to SDS-PAGE gels (25 µl/lane) and probed with high affinity antibodies to Glut-1 (Cat Nos. GT11/GT12/GT13) using enhanced chemiluminescence (ECL) detection method. The intensity of bands will vary with other techniques and source of antibodies. Actual volume of this solution/per application must be determined by the user and adjusted accordingly. This products has given one major band with our antibodies at about 42-45 kDa. The actual number of bands will depend upon the quality of antibodies, antibody concentration, sample composition and concentration, detection method, and background.

Lyophilized products should be reconstituted in 100 µl water and gently mixed for 15 min at room temp. All peptide/antibody received in solution or reconstituted from Lyophilized vials should be stored frozen at –20°C or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 µg/ml for affinity pure antibody using Chemiluminescence technique). This antibody identified a major band at about 42-45 kDa using our ready-to-use Glut-1 positive control (Cat. No. GT11-C) form purified rat brain membranes in Western blotting (1).

ELISA: Control peptide can be used to coat ELISA plates at 1 µg/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 µg/ml for affinity pure).

Histochemistry & Immunofluorescence: we recommend the use of affinity purified antibody at 2-10 µg/ml on frozen sections of rat tissues (2).

Immuoprecipitation: use at 5-10 µl neat serum per 100 µg of rat brain membranes or 1-10 µg affinity pure.

Specificity & Cross-reactivity

This antibody has detected Glut-1 from human (RBC) and rabbit brain and mouse 3T3 L1 fibroblast and rat brain and adipocytes. It also recognizes the Hep G2 type transporter, and Glut-1 from Caco-2 cells (3). The Peptide sequence used for antibody production is 100% identical in mouse, rat, human, rabbit, bovine, and pig. Chicken Glut 1has about 90% homology (9/12 aa). Antibody crossreactivity in various species is not established. Control peptide, because of its low mol size (<3 Kda), is not recommended for Western. It should be used in ELISA, dot blot, and for antibody blocking to confirm specificity of antibodies.

References:

1. Haspel et al., (1986) J. Biol. Chem. 263, 398-403; Birnbaum, et al., (1986) 83, 5784-5788. 2. Piper et al., (1991) Am. J. Physiol. 260, C570-C580. 3. Harris et al. (1992) Proc. Natl. Acad. Sci. 89, 7556-7560.; see reviews by Baldwin, SA (1993) Biochim. Biophys. Acta 1154, 17-49; Mueckler, M (1994) Eur. J. Biochem. 219, 713-725.

"**Neat Antisera**" are the unpurified antiserum and it is suitable for ELISA and Western.
"Affinity pure" antibodies have been over the antigen-affinity column and recommended for immunohistochemical applications.
"Control peptides" can not be used for Western as they are very short peptides. They are intended for ELISA or antibody competition studies.