R&D Ab
Research & Diagnostic Antibodies
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# IgG Fraction of Rabbit Anti-Nicotinic β2 Receptor Sera

AS-5646G Lot # 9429

This IgG fraction was prepared by precipitation, dialysis and chromatography from a pool of antisera that were raised in rabbits which were immunized with a peptide analogue of the carboxyl terminal of the nicotinic β2 receptor (residues #493-502) attached onto a carrier protein. The whole antisera have been shown to be specific for the COOH terminal of nicotinic β2 receptor and have been characterized by western blotting, ELISA, and cell staining techniques. This IgG fraction is suitable for immunocytochemical and western immunoblotting detection of the receptor. Dilute the lyophilized antibody with 0.1 ml of 10 mg/ml BSA in PBS for the equivalent concentration in whole antiserum, or with additional buffer for more dilute antibody. This vial contains 2.15 mg of purified IgG.

## Antiserum Specificity

| Polypeptide | % Cross Reactivity |
|---|---|
| Nicotinic β2 receptor (493-502) | 100 |
| Nicotinic β2 receptor | ~90 |
| Nicotinic α3 receptor | 0 |
| Nicotinic α4 receptor | 0 |
| Nicotinic α5 receptor | 0 |
| Nicotinic α7 receptor | 0 |
| Nicotinic β3 receptor | 0 |
| Nicotinic β4 receptor | 0 |

## Immunocytochemical Staining

The pooled antisera have been found to stain fixed cells known to express the nicotinic β2 receptor using ABC techniques at a concentration of 20 μg per ml. Cells and tissues should be fixed using PLP – see our FAQ page at www.RDAbs.com for information.

## Western Immunoblot

Western immunoblots using tissue homogenates have been successful at a concentration of 25 μg per ml and yield a single band at 60 kDa.

## Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the IgG fraction after diluting to at least 25 μg per ml (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash for 5 minutes x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.