R&D® Ab

Research & Diagnostic Antibodies

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Rabbit Anti-Nicotinic α4 Receptor Sera

AS-5616S Lot # 9407

These antisera were raised in rabbits which were immunized with a peptide analogue of the carboxyl terminal of the nicotinic α4 receptor (residues #620-627) attached onto a carrier protein. The antisera have been shown to be specific for the carboxyl terminal of the nicotinic α4 receptor, have been characterized by western blotting, ELISA, and cell staining techniques, and are 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 of whole antiserum, or with additional buffer for more dilute antibody.

Antiserum Specificity

Polypeptide	% Cross Reactivity
Nicotinic α4 receptor (620-627)	100
Nicotinic α4 receptor	~80
Nicotinic α3 receptor	0
Nicotinic α5 receptor	0
Nicotinic α7 receptor	0
Nicotinic β2 receptor	0
Nicotinic β3 receptor	0
Nicotinic β4 receptor	0

Immunocytochemical Staining

The pooled antisera have been found to stain PLP fixed cells known to express the nicotinic α4 receptor using ABC techniques at a 1:200 dilution. For information on PLP fixative see the FAQ page on our web site at www.RDAbs.com

Western Immunoblot

Western immunoblots using tissue homogenates have been successful at a 1:800 dilution and yield a single band at 75 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 4% normal goat serum using TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the antibody after preparing a 1:800 dilution (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.