

**Rabbit Anti-Nicotinic  $\alpha$ 5 Receptor Sera**

AS-5621S

Lot # 9410

These antisera were raised in rabbits which were immunized with a peptide analogue of the carboxyl terminal of the nicotinic  $\alpha$ 5 receptor (residues #460-468) attached onto a carrier protein. The antisera have been shown to be specific for the COOH terminal of nicotinic  $\alpha$ 5 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**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
Nicotinic $\alpha$ 5 receptor (460-468)	100
Nicotinic $\alpha$ 5 receptor	~95
Nicotinic $\alpha$ 3 receptor	0
Nicotinic $\alpha$ 4 receptor	0
Nicotinic $\alpha$ 7 receptor	0
Nicotinic $\beta$ 2 receptor	0
Nicotinic $\beta$ 3 receptor	0
Nicotinic $\beta$ 4 receptor	0

**Immunocytochemical Staining**

The pooled antisera have been found to stain PLP fixed cells known to express the nicotinic  $\alpha$ 5 receptor using ABC techniques at a 1:400 dilution. For information on PLP fixative see the FAQ page on our web site at [www.RDAbs.com](http://www.RDAbs.com)

**Western Immunoblot**

Western immunoblots using tissue homogenates have been successful at a 1:1600 dilution 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 as 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 1:1600 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.