

**IgG Fraction of Rabbit Anti-Nicotinic  $\alpha$ 3 Receptor Sera**

AS-5611G

Lot #10687

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  $\alpha$ 3 receptor (residues #466-474) attached onto a carrier protein. The whole antisera have been shown to be specific for the COOH terminal of nicotinic  $\alpha$ 3 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 1.11 mg of purified IgG.

**Antiserum Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
Nicotinic $\alpha$ 3 receptor (466-474)	100
Nicotinic $\alpha$ 3 receptor	~90
Nicotinic $\alpha$ 4 receptor	0
Nicotinic $\alpha$ 5 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 fixed cells known to express the nicotinic  $\alpha$ 3 receptor using ABC techniques at a concentration of 20  $\mu$ g per ml. Cell and tissues should be fixed using PLP fixative – see our FAQ page at [www.RDAbs.com](http://www.RDAbs.com) for information.

**Western Immunoblot**

Western immunoblots using tissue homogenates have been successful at a concentration of 25  $\mu$ g per ml and yield a single band at 59 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 IgG fraction after diluting to at least 25  $\mu$ g 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.