

DESCRIPTION	
Species Reactivity	Rat
Specificity	Detects rat Agrin in ELISAs and Western blots. In sandwich immunoassays, less than 0.1% cross-reactivity with recombinant rat (rr) GDNF, rrIL-1 α , and rrL-Selectin is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf21-derived recombinant rat Agrin Ala1153-Pro1959 (Pro1788-Ser1798 del) Accession # P25304
Endotoxin Level	<0.10 EU per 1 μ g of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 μ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μ m filtered solution in PBS.

APPLICATIONS		
Please Note: Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.		
	Recommended Concentration	Sample
Western Blot	0.1 μ g/mL	Recombinant Rat Agrin (Catalog # 550-AG)
Immunohistochemistry	5-15 μ g/mL	See Below
Rat Agrin Sandwich Immunoassay		Reagent
ELISA Capture	0.2-0.8 μ g/mL	Rat Agrin Antibody (Catalog # AF550)
ELISA Detection	0.1-0.4 μ g/mL	Rat Agrin Biotinylated Antibody (Catalog # BAF550)
Standard		Recombinant Rat Agrin (Catalog # 550-AG)
Neutralization	Measured by its ability to neutralize Agrin-induced acetylcholine receptor clustering on chick myotubes. The Neutralization Dose (ND ₅₀) is typically 0.001-0.004 μ g/mL in the presence of 0.016 μ g/mL Recombinant Rat Agrin.	

DATA	
<p>Neutralization</p> <p>Acetylcholine Receptor Clustering Induced by Agrin and Neutralization by Rat Agrin Antibody. Recombinant Rat Agrin (Catalog # 550-AG) induces acetylcholine receptor clustering on chick myotubes in a dose-dependent manner (orange line). Acetylcholine Receptor Clustering elicited by Recombinant Rat Agrin (0.016 μg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Rat Agrin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF550). The ND₅₀ is typically 0.001-0.004 μg/mL.</p>	<p>Immunohistochemistry</p> <p>Agrin in Mouse Kidney. Agrin was detected in perfusion fixed frozen sections of mouse kidney using Goat Anti-Rat Agrin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF550) at 0.1 μg/mL overnight at 4 °C. Tissue was stained using the Northern-Lights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to basement membrane of epithelial cells in tubules and endothelial cells in glomeruli. View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.</p>

PREPARATION AND STORAGE	
Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Agrin, a heparan sulfate proteoglycan, is a component of the synaptic basal lamina which promotes acetylcholine receptor (AChR) clustering on cultured myotubes and *in vivo*. This AChR clustering activity has been shown to be mediated via a receptor complex that includes a receptor-like tyrosine kinase specific to the skeletal muscle termed muscle-specific kinase (MuSK), and an as of yet unidentified myotube-specific accessory component.

Agrin contains a number of distinct domains. The N-terminal half of the molecule, which is responsible for the tight interaction with the extracellular matrix, has nine follistatin-like repeats that share homology to Kazal-type protease inhibitor domains. The C-terminal half, which by itself is sufficient for the AChR clustering activity, has four EGF-like repeats and three laminin globular G domains. Agrin exists in several isoforms which are generated by alternative splicing at multiple splicing sites in the C-terminal half. Some of these isoforms are expressed specifically in the nervous system while other isoforms are expressed in both neural and nonneural tissues. Dramatic differences in AChR clustering activities have been observed between the different isoforms. The highest activity is found to be associated with isoforms found exclusively in neural tissues that contain the four amino acids K-S-R-K at position # 1643-1646 and the eight amino acids E-L-T-N-E-I-P-A after Ser1779. Rat and chick Agrin share approximately 60% amino acid sequence identity. R&D Systems recombinant C-terminal half fragment of Agrin is a soluble secreted protein which has been found to have AChR clustering activity on chick myotubes.

References:

1. Kleiman, R.J. and L.F.Reichardt (1996) Cell **85**:461.
2. Glass, D. *et al.* (1996) Cell **85**:513.
3. Bowe, M. and J. Fallon (1995) Annu. Rev. Neurosci. **18**:443.