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RABBIT ANTI- AMYLOID OLIGOMER POLYCLONAL ANTIBODY

CATALOG NUMBER: AB9234

LOT NUMBER:

QUANTITY: 100 μL

SPECIFICITY: Amyloid oligomer. The antibody recognize all types of amyloid oligomers. The antibody appears to recognize a peptide backbone epitope that is common to amyloid oligomers, but is not found in native proteins, amyloidogenic monomer or mature amyloid fibrils. Amyloidogenic conformations of non-disease related proteins can be created by partial protein misfolding or denaturation. This antibody has been referred to as A11.

APPLICATIONS: Immunohistochemistry: 1:1,000-1:10,000 (see suggested protocol on back)
Immunoprecipitation: 1:1,000. Suggested cell lysis buffer is RIPA. Suggested capture agent is magnetic beads (Dynabeads). Known co-precipitatiing polypeptide: Amyloid beta, alpha synuclein oligomers.
ELISA (direct)
Optimal working dilutions must be determined by the end user.

SPECIES REACTIVITY: Human to yeast. Other species have not been tested.

FORMAT: Rabbit serum.

PRESENTATION: Liquid. Contains no preservative.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles.

REFERENCES:

1) R. Kayed, E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman and C.G. Glabe, *Science* (2003) **300**:486-9.
2) S. Oddo, A. Caccamo, J.D. Shepherd, M.P. Murphy, T.E. Golde, R. Kayed, R. Metherate, M.P. Mattson, Y. Akbari and F.M. LaFerla, *Neuron* (2003) **39**:409-21.
3) A. Sanbe, H. Osinska, J.E. Saffitz, C.G. Glabe, R. Kayed, A. Maloyan and J. Robbins, *Proc Natl Acad Sci U S A* (2004) **101**:10132-6.
4) J. Shorter and S. Lindquist, *Science* (2004) **304**:1793-7.
5) C.G. Glabe, *Trends Biochem Sci* (2004) **29**:542-7.
6) Lesne, S., et al., *Nature* (2006) **440**:352-357.

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Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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SUGGESTED IMMUNOHISTOCHEMISTRY PROTOCOL

Buffers:
Tris - 0.1M Tris, 0.85% saline, pH 7.4-7.6
Tris A – 0.1M Tris, 0.85% saline, pH 7.4-7.6, 0.1% Triton X-100
Tris B - 0.1M Tris, 0.85% saline, pH 7.4-7.6, 0.1% Triton X-100, 2.0% bovine serum albumin

Pretreatment
For paraffin slides – defat (xylene) and rehydrate into Tris
Pap pen around section or use coverslips
Incubate in 3% H_20_2, 10% methanol in Tris solution for 30 minutes
Wash in Tris – 5 min
Wash in Tris A – 15 min
Wash in Tris B – 30 min

Anti-Amyloid oligomer (AB9234)
Prior to use centrifuge the undiluted AB9234 antibody and pull from the top layer. Dilute the AB9234 in Tris B solution. The antibody is expected to work well between 1:1,000-1:10,000 but will need optimization for paraffin sections. Incubate overnight at room temperature on orbit shaker.
Wash 2 x 5 min in Tris A
Wash 15 min in Tris B
Incubate in biotinylated anti-rabbit IgG (for example Chemicon Cat. No. AP132B) for 1 hour at room temp
Wash 2 x 5 min in Tris A
Proceed with avidin-biotin detection system.

Potential problems:
The antibody tends to aggregate easily leaving “clumps” of DAB positive product on the top of the tissue – centrifuging the antibody prior to dilution helps this somewhat. It has been found that formic acid pretreatment does not improve the immunostaining substantially but this is another pretreatment that may help improve the signal (90% formic acid for 4 min). Other pretreatments may be necessary (ie. Boiling, microwaving, antigen retrieval solutions) but have not yet been evaluted.

Cryostat sections : 4% paraformaldehyde fixed tissue, embedded in OCT and frozen at –80°C.
Several different protocols for antigen retrieval should be tried. It is recommended that you try at least 3 and settle on the one works that works best for you.
Let frozen sections melt and dry at RT for 5-15 min before processing them for immunofluorescence.

Parafin sections :10% buffered formalin fixed tissue – 4-5 μm thick, dried on a warm plate for 2-6 hrs, then deparafinized: xylene 3X, EtOH 100% - 2X, then EtOH 95%, 70%, 50% - 1X each, dH_2O – 1X – 3 min each change.

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SUGGESTED IMMUNOHISTOCHEMISTRY PROTOCOL (cont)

Processing for immunofluorescence

1) PBS treat for 5 min.
2) Antigen retrieval (**AR**) solution used for AB9234 Ab - 0.1M glycine/PBS, pH 3.5
Place slides in plastic container with a plastic rack filled with AR solution; fill the extra spaces on the rack with empty slides. Put in microwave oven and bring to boiling on high power (takes about 1.5 min), then set your microwave oven on a lower power for 30- 35 min (30 min for cryosections, 35 min. for paraffin sections). The power setting is adjusted so that the oven cycles on and off every 20-30 seconds and the solution boils about 5-10 sec. each cycle without any liquid runover. We have found large variations in the success of this method that appear to be microwave dependent! Cool down in the same bath, at RT 30 - 45 min.
3) PBS – 2 X 5 min.
4) Blocking – 1 hr, RT (Blocking solution (1%BSA, 0.1% cold water fish skin gelatin, 0.1% Tween 20 in PBS with 0.05% sodium azide).
5) AB9234 diluted appropriately (diluted in a 1:1 mixture of blocking solution and PBS (1:200)) in refrigerator, overnight. Use Cover Well chambers and a humid container.
6) PBS - 2-3 x 5 min.
7) Blocking – 20 min.
8) Proceed with secondary detection (Alexa 488 or Alexa 568 work well).

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