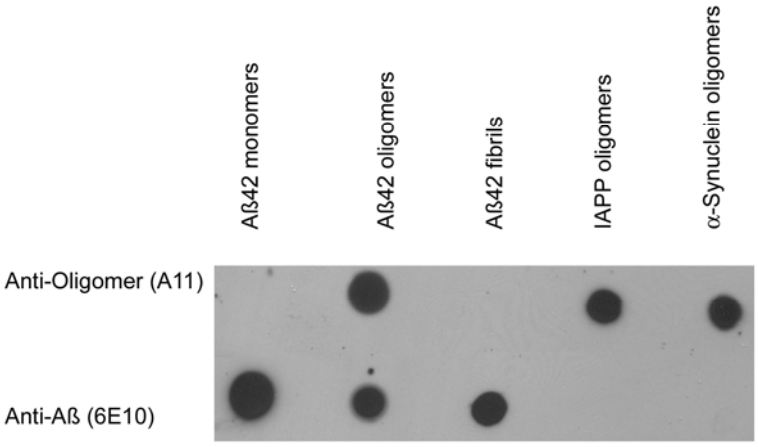
# Rabbit (polyclonal) Anti-Oligomer Antibody (A11)
Unconjugated

## PRODUCT ANALYSIS SHEET

| | |
|---|---|
| **Catalog Number:** | AHB0052 |
| **Lot Number:** | See product label |
| **Quantity/Volume:** | 50 µg/50 µL (Antibody concentration: 1.0 mg/mL) |
| **Form of Antibody:** | Purified rabbit immunoglobulins in phosphate buffered saline, pH 7.4. |
| **Preservation:** | 0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly). |
| **Purification:** | Purified by Protein A affinity chromatography. |
| **Immunogen:** | Synthetic molecular mimic of soluble oligomers (1). |
| **Specificity:** | This antibody recognizes amino acid sequence-independent oligomers of proteins or peptides. A11 does not recognize monomers or mature fibers of proteins or peptides. For example, A11 reacts with soluble Aβ40 oligomers and does not react with soluble low molecular weight Aβ40 or Aβ40 fibrils (1). A11 recognizes oligomeric species of several other amyloidogenic polypeptides including Aβ42, human insulin, prion, polyglutamine, lysozyme, α-synuclein and yeast prion Sup35 (1, 2).<br>Many degenerative diseases are known to be related to the accumulation of misfolded proteins as amyloid fibrils. These include the amyloid-β peptide plaques and tau neurofibrillary tangles in senile plaques of Alzheimer's disease, the deposition of α-synuclein in the Lewy bodies of Parkinson's disease and accumulation of polyglutamine-containing aggregates in Huntington's disease, etc. Soluble amyloid oligomers are considered as the principal pathogenic species which play an important role in the formation of amyloid fibrils and, therefore, are involved in the pathogenesis of the many neurodegenerative diseases. This anti-oligomer antibody provides a facile means of assessing the significance of oligomers in disease pathogenesis. |
| **Species Reactivity:** | Not species-specific. Expected to react with many species including human, mouse and rat. |
| **Applications:** | This antibody is suitable for use in ELISA, dot-blotting, immunohistochemistry and neutralizing assay (1-3). It has not been qualified for Western blot analysis. This product is only to be used and/or sold for use as a research reagent. With the sale of this product, a research use only license is conveyed to the purchaser. This product is not licensed for: (A) use as a diagnostic, (B) use as a therapeutic, and/or (C) use for drug screening to directly identify small molecule products for the prevention or treatment of human disease. |
| **Suggested Working Dilutions:** | The recommended concentration for ELISA and dot blotting is 0.1-1.0 µg/mL. For immunohistochemistry, 1-5 µg/mL is recommended. The optimal antibody concentration should be determined for each specific application. |
| **Recommended Controls:** | Oligomeric Aβ42 as a positive control; monomeric and fibrillar Aβ42 as negative controls. |

**This product is for research use only. Not for use in diagnostic procedures.**

**Storage:** Store at 2-8°C. For long term storage, apportion into working aliquots and store at -20°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.




**Specificity of anti-oligomer (A11) antibody.** Dot blot assay: One microgram aliquots of soluble Aβ42 monomers, Aβ42 oligomers, Aβ42 fibrils, islet amyloid polypeptide (IAPP) oligomers or α-synuclein oligomers were applied to a nitrocellulose membrane and probed with rabbit anti-oligomer antibody (Catalog no. AHB0052) (top row) or with mouse anti-Aβ42 [1-16] (bottom row). The anti-oligomer antibody recognizes all types of oligomers, but no monomers or fibrils.

**References:**


Kayed, R., et al. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486-489.

Shorter, J. and S. Lindquist (2004) Hsp104 catalyzes formation and elimination of self-replicating Sup35 prion conformers. Science 304:1793-1797.

Sanbe, A., et al. (2004) Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. Proc. Nat'l. Acad. Sci. USA 101:10132-10136.

Kayed, R., et al. (2004) Permeabilization of lipid bilayers is a common conformation dependent activity of soluble amyloid oligomers in protein mis-folding diseases. J. Biol. Chem. 279:46363-46366.

Glabe, C.G. (2004) Conformation-dependent antibodies target diseases of protein misfolding. Trends Biochem. Sci. 29:542-547.


**Related Products:**

| | | |
|---|---|---|
| Human Aβ40 ELISA kit | Cat. # | KHB3481/2 |
| Human Aβ42 ELISA kit | Cat. # | KHB3441/2 |
| Human APP ELISA | Cat. # | KHB0051 |
| Rabbit (polyclonal) Anti-Aβ, N-terminal, antibody | Cat. # | 44-338-100/44-338-50 |
| Rabbit (polyclonal) Anti-Aβ42, cleavage specific antibody | Cat. # | 44-344 |
| Rabbit (polyclonal) Anti-Aβ43, cleavage specific antibody | Cat. # | 44-340 |
| Mouse anti-human APP antibody (clone: mAbP2-1) | Cat. # | 44-100 |
| Human Tau (total) ELISA | Cat. # | KHB0041/2 |
| Human Tau [pS396] ELISA | Cat. # | KHB7031 |
| Human α-synuclein ELISA | Cat. # | KHB0061 |




www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com



PI AHB0052 (Rev 10/08) DCC-08-1089

**PROCEDURES:**

**Dot Blotting Procedure (Reference 1)**

1. In preparing samples, avoid conditions and methods that denature proteins or cause protein misfolding, such as cavitation, foaming, excessive heat, protein denaturants, organic solvents, denaturing detergents, oxidation, etc.
2. Divide nitrocellulose membrane into 1 cm grid lines and spot 1-3 µL of each sample onto the membrane. Spot positive and negative controls. Allow spots to air dry.
3. Block membrane in 10% non-fat dry milk TBST solution (formulation presented below) at 4°C for 1 hour to overnight.
4. Wash membrane 3 times in 1x TBST for 5 minutes.
5. Dilute primary antibody to 0.5-1 µg/mL in 5% non-fat dry milk TBST.
6. Cover membrane with primary antibody solution and incubate with gentle shaking for 1 hour.
7. Wash 3 times in TBST for 5 minutes.
8. Dilute secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (BioSource Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (BioSource Cat. # ALI4404), according to manufacturer's recommendations (usually 0.1 µg/mL) in 5% non-fat dry milk in TBST.
9. Cover membrane with secondary antibody solution and incubate with gentle shaking for 1 hour.
10. Wash 3 times in TBST for 5 minutes.
11. Add substrate solution according to manufacturer's recommendation and develop image.

**10x TBST (4 liters)**
320 g NaCl
96.8 g Tris
4 mL Tween 20
deionized $H_20$ to 4 liters
pH 7.6
Dilute 1:10 for use

**10% Non-fat dry milk in TBST**
10 g non-fat dry milk
fill to 100 mL with 1x TBST

**5% Non-fat dry milk in TBST**
5 g Non-fat dry milk
fill to 100 mL with 1x TBST

**Immunofluorescence Procedures**

A: Free floating sections. (Reference 1) Brains were immersion fixed in 10% buffered formalin for 48-72 hours and the hippocampus with entorhinal cortex was dissected and stored in phosphate buffered saline (PBS) with 0.05% sodium azide at 4°C until used. Blocks were stored in PBS with 0.05% sodium azide at 4°C. Free-floating 50 µm thick serial sections were subsequently collected using a vibratome. Serial free-floating sections were pretreated with 90% formic acid for 4 minutes. Sections were subsequently incubated in anti-oligomer (A11) antibody (1-5 µg/mL) overnight at room temperature. For confocal microscopy, anti-oligomer antibody was visualized using fluorescent conjugated secondary antibody. Sections were coverslipped using Vectashield mounting media (Vector Laboratories, Inc., Burlingame, CA).

B: Paraffin sections. (Reference 3) 10% buffered formalin fixed tissue. Paraffin sections 4-5 µm thick were prepared, dried on a warm plate for 2-6 hours, then deparafinized using the following series: xylene 3x, 100% EtOH 2x, then EtOH 95%, 70%, 50%, 1x each, 3 minutes each change, followed by distilled or deionized $H_2O$ for 3 minutes.

1. Wash the slides in PBS for 5 minutes.
2. Place slides in plastic container with a plastic rack filled with Antigen Retrieval solution (0.1 M glycine/PBS, pH 3.5); fill the extra spaces on the rack with empty slides. Place the slides in a microwave oven and bring to boiling on high power, a process which requires about 1.5 minutes, then set the microwave oven on a lower power for 30-35 minutes (30 minutes for cryosections, 35 minutes for paraffin sections). The power setting should be adjusted so that the oven cycles on and off every 20-30 seconds and the solution boils about 5-10 seconds each cycle without any liquid runover. It is important to note that we have found large variations in the success of this method that appear to be microwave dependent; therefore, some optimization of the procedure may be required with each microwave oven. Allow the slides to cool in the same bath, at RT 30-45 minutes.
3. Wash the slides in PBS, 2 times, 5 minutes each.
4. Block the slides for one hour at RT using a blocking solution containing 1% BSA, 0.1% cold water fish skin gelatin, 0.1% Tween 20 in PBS with 0.05% sodium azide.
5. Add the anti-oligomer antibody (diluted in a 1:1 mixture of blocking solution and PBS to a final concentration of 1-5 µg/mL). Allow to incubate overnight in a refrigerator at 4°C. Use Cover Well chambers and a humid container.
6. Wash the slides in PBS, 3 times, 5 minutes each.
7. Block the slides for 20 minutes at RT.
8. React the slides with an appropriate secondary antibody (Alexa 488 or Alexa 568 conjugated) diluted in a 1:1 mixture of PBS and blocking solution, for 1 hour at RT in dark according to manufacturer's recommendations.
9. Wash the slides in PBS, 3 times, five minutes each.
10. Block the slides for 20 minutes at RT.



www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

PI AHB0052 (Rev 10/08) DCC-08-1089

11. React the slides with phalloidin/Alexa 568 or 488 (1:100) + nuclear stain (depending on your lasers ) for 15 minutes in the dark.
12. Mount the slides in Vecta Shield, Hard Set. Allow the slides to set for at least 30 minutes in the dark at RT. Store slides at 4°C.

**ELISA Procedure (Reference 1)**

1. In preparing samples, avoid conditions and methods that denature proteins or cause protein misfolding, such as cavitation, foaming, excessive heat, protein denaturants, organic solvents, denaturing detergents, oxidation, etc.
2. Add 100 µL of diluted samples (and 0.1 to 10 ng of monomer, oligomer and amyloid fibril standards) in coating buffer (0.1 M sodium bicarbonate, pH 9.6) to each well of a 96 well plate.
3. Incubate plate at 37°C for 2 hours.
4. Wash plate 3 times with PBS.
5. Add 200 µL of blocking buffer (10% ELISA grade BSA in TBST) to each well.
6. Incubate at 37°C for 1 hour.
7. Wash plate 3 times with PBS.
8. Dilute primary antibody to 1 µg/mL in 3% BSA in TBST.
9. Add 100 µL of primary antibody solution and incubate at 37°C for 1 hour.
10. Wash plate 3 times with PBS.
11. Dilute secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (BioSource Cat. # ALI4404), according to manfacturer's instructions.
12. Add 100 µL of secondary antibody and incubate at 37°C for 1 hour.
13. Wash plate 3 times with PBS.
14. Develop plate according to secondary antibody manufacturer's recommendation and read plate.