

IgG Fraction of Rabbit Anti- β -Amyloid(1-28) Serum

AS-1917

Lot # 7579

This IgG fraction was prepared by precipitation, dialysis and chromatography from an antiserum that was raised in a rabbit which was immunized with β -amyloid(1-28) covalently attached onto a carrier protein. The antiserum has been shown to be specific for the amino terminal of β -amyloid and has been characterized by western blotting, ELISA, and cell staining techniques. This IgG fraction is suitable for immunocytochemical, immunoprecipitation and western immunoblotting of β -amyloid. 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 2.2 mg of purified IgG.

Antiserum Specificity

Polypeptide	% Cross Reactivity
β -amyloid(1-28)	100
ACTH	0
ANP	0
Dynorphin A(1-13)	0
β -endorphin A(human)	0
PTH(1-84)	0

Immunocytochemical Staining

This IgG fraction has been found to stain β -amyloid using ABC techniques at a dilution of 1:5000.

Western Immunoblot

Western immunoblots have been successful at a dilution of 1:500.

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 in 1:5 diluted evaporated goat milk, using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:800 (Note: higher dilutions may be needed). Use 1% normal goat serum in 1:5 diluted evaporated goat milk as buffer for the primary antibody. Dilute the condensed goat milk with 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 1% normal goat serum in 1:5 diluted evaporated goat milk (use TBS/Tween-20 to dilute the goat milk). Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 3 and then soak the membrane overnight in a fairly large volume of TBS/Tween-20.
7. Develop color using the DAB reaction or the enhanced DAB reaction.