SYSY Antibodies

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# Oligo-Abeta-pE3

**Cat.No. 218 511; Monoclonal mouse antibody, 100 µg purified IgG (lyophilized)**

## Data Sheet

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|---|---|
| Reconstitution/ Storage | 100 µg purified IgG, lyophilized. Albumin and azide were added for stabilization.<br>For **reconstitution** add 100 µl $H_2O$ to get a 1mg/ml solution in PBS. Then aliquot and store at -20°C to -80°C until use.<br>Antibodies should be stored at +4°C when still lyophilized. Do not freeze!<br>For detailed information, see back of the data sheet. |
| Applications | **WB**: 1 : 500 (ECL detection) (see remarks)<br>**IP**: not tested yet<br>**ICC**: not tested yet<br>**IHC**: 1 : 100<br>**IHC-P (FFPE)**: 1 : 100 (see remarks)<br>**ELISA**: external data |
| Important note for users | The mouse monoclonal antibody clone 9D5, Cat. No. 218 511, is patented (patent application PCT/EP2011/002739). By purchasing this antibody the customer acquires rights to use this product for research purposes only. Any diagnostic and therapeutic in vitro or in vivo use is explicitly excluded. |
| Clone | 9D5 |
| Subtype | IgG2b (κ light chain) |
| Immunogen | Synthetic peptide corresponding to AA 3 to 38 from human Oligo-Abeta-pE3 (UniProt Id: P05067) |
| Reactivity | Reacts with: human (P05067), mouse (P12023).<br>Other species not tested yet. |
| Specificity | Recognizes specific oligomeric structures formed preferentially by Abeta-pE3. |
| Remarks | **WB**: We recommend the Invitrogen NativePAGE system in combination with PVDF blotting membranes. Boil membrane in PBS after blotting in PBS for 3min. This antibody has only been validated with synthetic peptides and not with complex protein samples. **Peptide preparation**: Synthetic Abeta peptides were monomerized in 70 % formic acid, and the solvent was evaporated in a speed-vac immediately. Prior to each experiment, peptides were dissolved in 0.3 % ammonia, underwent ultrasonic treatment, and were further diluted to an end concentration of 0.15 % ammonia.<br>**IHC-P (FFPE)**: Antigen retrieval with citrate buffer pH 6, followed by formic acid treatment, is required for chromogenic detection.<br>For chromogenic detection, an optimized AGR time of 20 minutes is recommended for best results. |

**TO BE USED IN VITRO / FOR RESEARCH ONLY**
**NOT TOXIC, NOT HAZARDOUS, NOT INFECTIOUS, NOT CONTAGIOUS**

## Background

Amyloid deposits, also called plaques, of Alzheimer's patients consist of several protein components like the **a**myloid **beta**-peptides (**Abeta**, **Aβ**) 1-40/42 and additional C- and N-terminally truncated and modified fragments. Very abundant are the isoaspartate (isoAsp)-Abeta and **p**yro**glu**tamyl (**pGlu**)-Abeta peptides. The latter are formed by cyclization of the N-terminal glutamate at position 3 or 11 catalyzed by glutaminyl cyclase (QC) resulting in very amyloidogenic and neurotxic variants of Abeta; **Abeta-pE3** and Abeta pE11.
In contrast to extracellular plaques that do not perfectly correlate with Alzheimer´s disease intraneuronal Abeta accumulation and vascular Abeta deposits have gained more and more evidence to be among the crucial factors responsible for progressive neuron loss.

## Selected References for 218 511

Identification of low molecular weight pyroglutamate A{beta} oligomers in Alzheimer disease: a novel tool for therapy and diagnosis.
Wirths O, Erck C, Martens H, Harmeier A, Geumann C, Jawhar S, Kumar S, Multhaup G, Walter J, Ingelsson M, Degerman-Gunnarsson M, et al.
The Journal of biological chemistry (2010) 28553: 41517-24. . **WB, IHC, ELISA**

Focusing the amyloid cascade hypothesis on N-truncated Abeta peptides as drug targets against Alzheimer's disease.
Bayer TA, Wirths O
Acta neuropathologica (2014) 1276: 787-801. . **IHC-P; tested species: human**

I716F AβPP mutation associates with the deposition of oligomeric pyroglutamate amyloid-β and α-synucleinopathy with Lewy bodies.
Sieczkowski E, Milenkovic I, Venkataramani V, Giera R, Ströbel T, Höftberger R, Liberski PP, Auff E, Wirths O, Bayer TA, Kovacs GG, et al.
Journal of Alzheimer's disease : JAD (2015) 441: 103-14. . **IHC; tested species: human**

Oxidative Stress during the Progression of β-Amyloid Pathology in the Neocortex of the Tg2576 Mouse Model of Alzheimer's Disease.
Porcellotti S, Fanelli F, Fracassi A, Sepe S, Cecconi F, Bernardi C, Cimini A, Cerù MP, Moreno S
Oxidative medicine and cellular longevity (2015) 2015: 967203. . **IHC**

Oligomeric pyroglutamate amyloid-β is present in microglia and a subfraction of vessels in patients with Alzheimer's disease: implications for immunotherapy.
Wirths O, Hillmann A, Pradier L, Härtig W, Bayer TA
Journal of Alzheimer's disease : JAD (2013) 354: 741-9. . **IHC**

Antibody 9D5 recognizes oligomeric pyroglutamate amyloid-β in a fraction of amyloid-β deposits in Alzheimer's disease without cross-reactivity with other protein aggregates.
Venkataramani V, Wirths O, Budka H, Härtig W, Kovacs GG, Bayer TA
Journal of Alzheimer's disease : JAD (2012) 292: 361-71. . **IHC**

Intraneuronal Aβ as a trigger for neuron loss: can this be translated into human pathology?
Bayer TA, Wirths O
Biochemical Society transactions (2011) 394: 857-61. .

## Selected General References

Pyroglutamate-Aβ 3 and 11 colocalize in amyloid plaques in Alzheimer's disease cerebral cortex with pyroglutamate-Aβ 11 forming the central core.
Sullivan CP et al. Neurosci. Lett. (2011) PubMed:22001577

Access the online factsheet including applicable protocols at https://sysy.com/product/218511 or scan the QR-code.

# FAQ - How should I store my antibody?

## Shipping Conditions

- All SYSY antibodies and control proteins/peptides are shipped lyophilized (vacuum freeze-dried). In this form, they remain stable without loss of quality at ambient temperatures for several weeks.

## Storage of Sealed Vials after Delivery

- **Unlabeled** and **biotin-labeled antibodies** and **control proteins** should be stored at **4°C** before reconstitution. **Do not freeze lyophilized antibodies**. Temperatures below 0°C may impair performance.
- **Fluorescence-labeled antibodies** should be reconstituted immediately upon receipt. Long-term storage of lyophilized fluorophore-conjugates may cause aggregation.
- **Control peptides** should be stored at -20°C before reconstitution.

## Long Term Storage after Reconstitution (General Considerations)

- **Do not use frost-free ("no-frost") freezers.** These units periodically warm to remove ice buildup, causing freeze–thaw cycles that can damage antibodies.
- Store vials in areas with minimal temperature fluctuation - preferably toward the back of the freezer, not on the door.
- Aliquot reconstituted antibodies and store at –20°C to –80°C.
- Avoid very small aliquots (<20 µL), as evaporation and adsorption to tube surfaces can reduce antibody concentration and activity.
- Use the smallest practical storage vial to minimize surface area.
- Adding glycerol to a final concentration of 50% prevents freezing at -20°C, allowing storage in liquid form and effectively avoiding freeze–thaw cycles.

## Product Specific Hints for Storage

### Control proteins / peptides

- Store at -20°C to -80°C

### Monoclonal Antibodies

- **Ascites and hybridoma supernatant:** Store at -20°C to -80°C
  Prolonged storage at 4°C is not recommended, as proteases present in ascites may degrade antibodies.
- **Purified IgG:** Store at -20°C to -80°C
  Adding a carrier protein (e.g., BSA) enhances long-term stability.
  Many SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

### Polyclonal Antibodies

- **Crude antisera:** Can be stored at 4°C with antimicrobials added, but -20°C to -80°C is preferred
- **Affinity-purified antibodies:** Less stable than antisera; store at -20°C to -80°C
  Adding a carrier protein such as BSA improves long-term stability.
  Most SYSY antibodies already contain carrier proteins - refer to the respective datasheet for details.

### Fluorescence-labeled Antibodies

- Store as a liquid with 1:1 (v/v) glycerol at -20°C, and protect from light exposure

# Avoid repeated freeze-thaw cycles for all antibodies!

# FAQ - How should I reconstitute my antibody?

## Reconstitution

- All purified SYSY antibodies are lyophilized from PBS. To reconstitute the antibody in PBS, add the volume of deionized water specified in the corresponding datasheet. If a larger final volume is desired, first add the recommended amount of water, then adjust with PBS and, if needed, add a stabilizing carrier protein (e.g., BSA) to a final concentration of 2%. Some SYSY antibodies already contain albumin; please take this into account before adding additional carrier protein.

For complete reconstitution, carefully remove the vial cap. After adding water, briefly vortex the solution. To collect the liquid at the bottom of the vial, place the vial inside a 50 ml centrifuge tube padded with paper and centrifuge briefly.

- If desired, small amounts of azide or thimerosal may be added to prevent microbial growth. This is particularly recommended when storing an aliquot at 4°C.
- After reconstitution of fluorescence-labeled antibodies, add glycerol 1:1 (v/v) to achieve a final concentration of 50%. This prevents freezing at –20°C and keeps the antibody in liquid form, effectively avoiding freeze–thaw cycles.
- Glycerol may also be added to unlabeled primary antibodies as a general measure to prevent freeze–thaw damage.
- For further guidance, please refer to our **storage tips** and recommendations for reconstituted antibodies, control peptides, and control proteins.