

Mouse alpha Synuclein protein (active, Pre-Formed Fibrils)

Cat. No. GTX17671-pro

Applications Functional Assay

Species Mouse

References (1)

Package

200 µg, 100 µg

PRODUCT

Summary Active Mouse Recombinant Alpha Synuclein Pre-formed Fibrils (Type 1)

Applications

Application Note

Endogenous alpha-synuclein phosphorylation. 100 µM alpha synuclein protein monomer (GTX17670-pro) seeded with 10 nM alpha synuclein protein PFF (GTX17671-pro) in 25 µM Thioflavin T (PBS pH 7.4, 100 µl reaction volume) generated an increased fluorescence intensity after incubation at 37°C with shaking at 600 rpm for 24 hours. Fluorescence was measured by excitation at 450 nm and emission at 485 nm on a Molecular Devices Gemini XPS microplate reader.

*For best results, sonicate immediately prior to use.

Properties

Form Liquid

Buffer PBS

Preservative No preservatives

Storage Store as concentrated solution. Aliquot and store at -80°C. Avoid freeze-thaw cycles.

Concentration Batch dependent (Please refer to the vial label for the specific concentration.)

Region/Sequence Full-length without tagged; MDVFMKGLSK AKEGVVAAA KTKQGVAAEA GKTKEGVLYV GSKTKEGVWH GVTTVAEKT
EQVTNVGGAV VTGVTAVAQK TVEGAGNIAA ATGFVKKDQM GKGEEGYPQE GILEDMPVDP GSEAYEMPSE EGYQDYEP

Expression System E. coli

Purification Purified by ion-exchange chromatography

Purity > 95% by SDS-PAGE

Conjugation Unconjugated

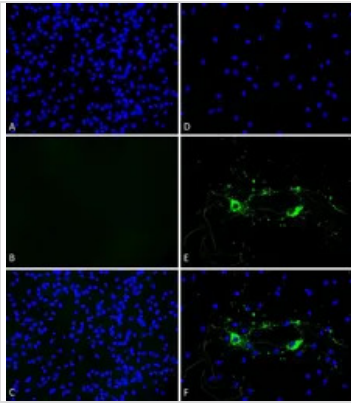
Note

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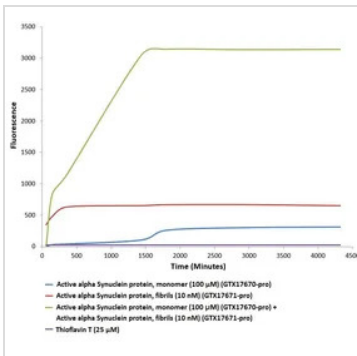
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DATA IMAGES

GTx17671-pro Functional Assay Image

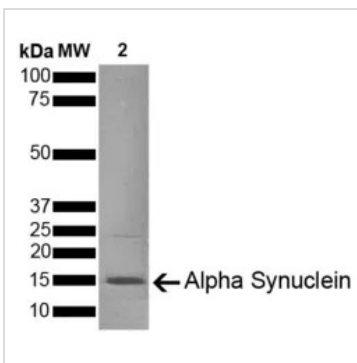
Primary rat hippocampal neurons (DIV16) show lewy body inclusion formation and loss of cells when treated with active mouse Alpha Synuclein Protein Preformed Fibrils (GTx17671-pro) at 4 $\mu\text{g}/\text{ml}$ (D-F) on DIV12, but not when treated with a control (A-C). Tissue: Primary hippocampal neurons. Species: Sprague-Dawley rat. Fixation: 3% formaldehyde from PFA for 20 min. Blocker: 1:1 PBS:LiCOR Odyssey Block (LiCOR, 927-40010) and 30 mL/mL of 0.1% triton-X 100 for 30 min. Primary Antibody: Mouse anti-pSer129 Antibody (1:1000) and Rabbit anti-pSer129 (1:800) for 24 hours at 4°C. Secondary Antibody: ATTO 546 Donkey Anti-Mouse (1:700) and ATTO 488 Donkey Anti-Rabbit (1:700) for 1 hour at RT (composite green). Counterstain: Hoechst (blue) nuclear stain at 1:3000 for 1 hour at RT. Localization: Lewy body inclusions. Magnification: 20x.


GTx17671-pro Functional Assay Image

Immunohistochemistry analysis of rat brain injected with active mouse alpha synuclein PFFs (GTx17671-pro). Species: Female Sprague-Dawley Rat. Rat was injected with 2 μL active mouse alpha synuclein PFFs (GTx17671-pro) in each of 2 injection sites: AP+1.6, ML+2.4, DV-4.2 from skull; and AP-1.4, ML+0.2, DV-2.8 from skull. 30 days post-injection. Fixation: Saline perfusion followed by 4% PFA fixation for 48 hrs. Secondary Antibody: Biotin-SP Donkey Anti-Rabbit IgG (H+L) at 1:500 for 2 hours in cold room with shaking. ABC signal amplification, DAB staining. Magnification: 20X. Alpha synuclein pathology is seen in the periform/insular cortex and the cingulate cortex on both the same (ipsi) and opposite (contra) sides as the injection sites.


GTx17671-pro Image

Active alpha synuclein preformed fibrils (GTx17671-pro) seed the formation of new alpha synuclein fibrils from the pool of alpha synuclein monomers (GTx17670-pro). Thioflavin T is a fluorescent dye that binds to beta sheet-rich structures, such as those in alpha synuclein fibrils. Upon binding, the emission spectrum of the dye experiences a red-shift, and increased fluorescence intensity. Thioflavin T emission curves show increased fluorescence (correlated to alpha synuclein protein aggregation) over time when 10 nM of active alpha synuclein preformed fibrils (GTx17671-pro) is combined with 100 μM of alpha synuclein monomer (GTx17670-pro), as compared to active alpha synuclein preformed fibrils (GTx17671-pro) or alpha Synuclein monomer (GTx17670-pro) alone. Thioflavin T $\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 485 \text{ nm}$.


GTx17671-pro Image

SDS-PAGE of ~14 kDa active Mouse alpha Synuclein protein (active, fibrils) (GTx17671-pro). Lane 1: Molecular Weight Ladder (MW). Lane 2: active Mouse alpha Synuclein protein (active, fibrils) (2 μg) (GTx17671-pro).



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