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FRONTIERS IN LIFE SCIENCE

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Anti-SOD1 (ALS-related mutants) Cocktail

Human, Rat-Mono (MS785/MS27)

Catalog No. FDV-0021A, FDV-0021B, FDV-0021C

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Product Background

SOD1 (Cu/Zn superoxide dismutase) is a ubiquitously expressed protein and generally forms homo-dimer. Its primary function is thought to be as a cytosolic and mitochondrial antioxidant enzyme, converting superoxide to molecular oxygen and hydrogen peroxide. Many lines of evidence suggest that SOD1 is one of the major causative genes of Amyotrophic Lateral Sclerosis (ALS), which is a fatal adult-onset neurodegenerative disease. Although nearly 90% of ALS cases are caused sporadically, around 10% of ALS cases are familial and of the familial ALS ~20% are caused by genetic mutation of SOD1. Over 100 mutations in SOD1 have been reported so far, and transgenic mice bearing some mutant SOD1s such as G93A and A4V are frequently used as ALS model animals. However, pathophysiological functions of mutant SOD1 are still unclear. Elucidation of the causal relationship between mutation of SOD1 and ALS is a topic of neuropathology to develop medicines of SOD1-related ALS pathogenesis.

Recent progresses show that SOD1 mutant proteins causes ALS through a gain of toxic function. Prof. Ichijo and co-workers reported almost all SOD1 mutants cause a similar conformational change, and acquire neural toxicity through a binding to Derlin-1, an ER-associated degradation (ERAD) machinery protein[Ref.1]. They also developed the novel rat monoclonal anti-SOD1 antibodies, clone MS785 and MS27, which specifically bind to conformationally altered SOD1 mutants, not detecting wild type SOD1 homo-dimer (Fig.1). Although both clones are succeeded in specifically detecting over 100 SOD1 mutants[Ref.2], these antibodies failed to detect some specific mutants which have mutation located on each epitope region. Anti-SOD1 (ALS-related mutants) Cocktail is a cocktail of MS785 and MS27 and compensates the above problem. This product is a powerful tool to investigate pathophysiological roles of SOD1 mutants in ALS patients.

ALS is considered as an ER-stress disease. Zinc-deficiency is one of the causes of ER-stress and depletion of zinc from SOD1 induces conformational change dramatically. Under the zinc-deficient condition, endogenous wild-type SOD1 takes the conformation similar with ALS-related mutants. Actually both MS785 and MS27 could recognize wild-type SOD1 under the zinc-deficiency specifically. These antibodies also powerful tools to investigate zinc-related ER-stress research.

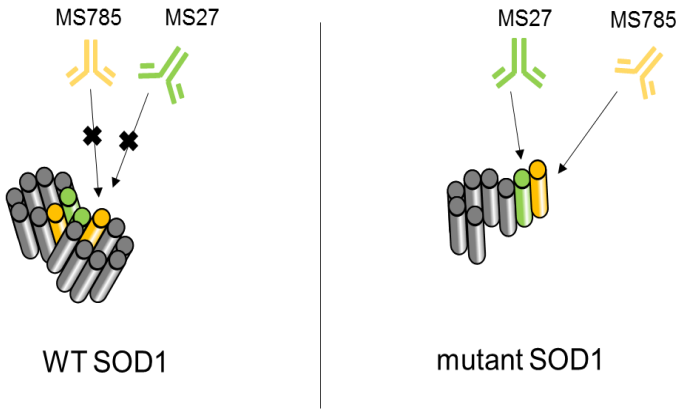

Figure 1. SOD1 recognition by MS785 and MS27

Description

Catalog Number and product size :
FDV-0021A (100 µg of MS785/MS27 cocktail; 50 µg each)
FDV-0021B (100 µg of MS785)
FDV-0021C (100 µg of MS27)
Concentration : 0.5 mg/ml
Volume : 200 µL
Formulation : 1x Phosphate Buffered Saline (PBS) containing 50% glycerol
Host Species and Clonality : Rat monoclonal
Isotype and Subclass : Clone MS785 IgG2b/κ, Clone MS27 IgG2a/κ
Purification : Protein G Purified
Lot Number : see vial label
Specificity : Clone MS785 human SOD1 8-14 amino acids
Clone MS27 human SOD1 30-40 amino acids
Storage : -20°C (Avoid repeated freeze-thaw cycles)

Application

- Immunoprecipitation to detect mutant SOD1s
- Immunocytochemistry and immunohistochemistry to detect mutant SOD1s
- ELISA to detect mutant SOD1s
- Western blotting to detect both mutant and wild-type SOD1
- Immunoprecipitation to detect wild type SOD1 under the zinc-related ER-stress

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How to use

- Immunoprecipitation to detect SOD1 mutants

Target : SOD1 mutants under native condition
Recommended amount : 1-5 µg/sample
Protocol example

1. Cells or tissues are lysed by non-denaturing lysis buffers such as RIPA buffer, 1% TritonX in TBS etc.
2. Add 1-5 ug of antibodies into the lysate and incubate for 1-10 hours.
   * NOTE:
   Amount of antibody and incubation time depend on samples, volume and experiments.
   Please optimize the condition for your experiments.
3. Add appropriate volume of Protein G-conjugated beads and incubate for 1-2 hours.
   * NOTE:
   Rat IgGs have weak binding affinity for Protein A. The use of Protein G is recommended.
4. Wash beads with appropriate wash buffers, ex. PBS containing 0.1% TritonX100, several times.
5. Elute binding proteins by SDS-PAGE sample buffer with heat or acidic buffers

Trouble shooting :
MS785 and MS27 could recognize not only mutants but also denatured SOD1. Please keep non-denatured condition and avoid to use high concentration of SDS or the other denaturing compounds.

- Detection of zinc-deficient ER-stress by immunoprecipitation

Target : Wild-type SOD1 under the zinc-deficient ER-stress

1. Cultured cells are treated with zinc-chelater TPEN (ex. 10 μM) for over 2 hours or are cultured in serum-free medium for over 24 hours to induce ER-stress.
2. Cells are lysed by appropriate non-denaturing lysis buffer
3. Add 1-5 μg of antibodies into lysate and incubate for 1-10 hours.
4. Add appropriate volume of Protein G-conjugated beads and incubate for 1-2 hours.
5. Wash beads with appropriate wash buffer
6. Elute binding proteins by SDS-PAGE sample buffer with boil or acidic buffers

- Immunocytochemistry and immunohistochemistry

Target : SOD1 mutantns

Recommended conc. : 1 μg/ml (1/500 dilution) -5 μg/ml (1/100 dilution)

1. Cells were fixed with 4% paraformaldehyde and permealized with appropriate detergent containing buffer
2. Blocking with appropriate blocking reagents such as 5% BSA, for 1 hours
3. Add antibodies containing buffer and incubate for 1-12 hours
4. Add 2nd antibody and incubate for 1-2 hours
5. Observe cells

- ELISA

Target : SOD1 mutants

Both MS785 and MS27 are available in ELISA format with commercially available the other SOD1 antibodies. Detail ELISA protocol is described in Ref 2. Please refer Ref. 2

- Western blotting

Target : wild-type and mutant SOD1s

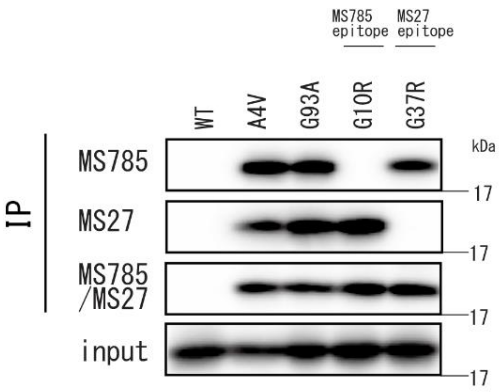
*Under the denaturing condition, MS785 and MS27 detect wild-type and mutant SOD1

Recommended conc. : Please optimize.

Application data

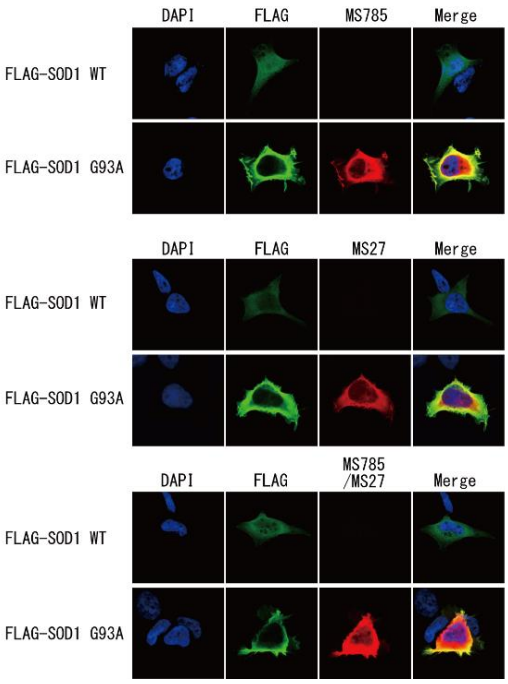
- Detection of SOD1 mutants by immunoprecipitation assay

SOD1 wild type or mutants-expressing HEK293 cells were lysed by 1% Triton X100/TBS buffer. After lysis, add MS785 (5 μg), MS27 (2 μg) or cocktail (1 μg) and incubate for 12 hours. Subsequently, SOD1-antibody complexes were captured by Protein G-beads. Neither MS785 nor MS27 single detected some specific mutants which have the mutation on the antibody's epitope. MS785/MS27 cocktail overcame this problem. Validated SOD1 mutants were listed in "**Appendix**".



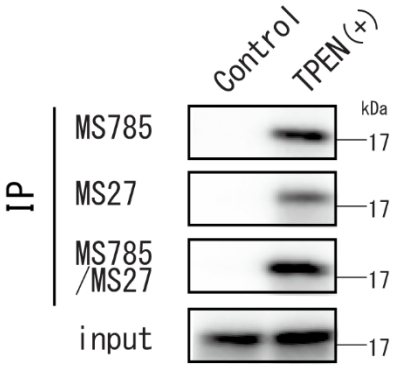
- Detection of SOD1 mutants by immunocytochemistry

Flag-tagged SOD1 wild type or G93A mutant-expressing HEK293 cells were fixed with 4% paraformaldehyde/PBS for 10 min at R.T. Subsequently, cells were incubated with PBS containing 0.2% Triton X-100 for 5 min and washed three times with PBS. After blocking with PBS containing 5% BSA, cells were incubated with primary antibodies (1 µg/ml MS785, MS27 or cocktail) for 12 hours at 4°C, with secondary antibody for 2 hours at R. T. sequentially. Cells were washed and stained with DAPI. Although anti-FLAG antibody clearly visualized either WT and G93A mutant, MS785, MS27 and MS785/MS27 cocktail only detected G93A mutant.



- Detection of endogenous SOD1 wild type under ER-stress

HEK293 cells were cultured in the presence and absence of 10 µM TPEN, a potent zinc-specific chelator, for 8 hours. After treatment, cells were lysed and immunoprecipitated with 1-5 µg of MS785, MS27 or cocktail. Subsequently, SOD1-antibody complexes were captured by Protein G-beads. Under the zinc-deficient ER-stress, these antibodies could recognize endogenous SOD1 wild type with mutant like conformation.



Reference

1. Fujisawa *et al.*, *Ann. Neurol.*, **72**, 739-749 (2012) A novel monoclonal antibody reveals a conformational alteration shared by amyotrophic lateral sclerosis-linked SOD1 mutants.
2. Fujisawa *et al.*, *Neurobiol. Dis.*, **82**, 478-486 (2015) A systematic immunoprecipitation approach reinforces the concept of common conformational alterations in amyotrophic lateral sclerosis-linked SOD1 mutants.

Appendix : Validated mutant SOD1

Mutants	MS785	MS27	Mix
WT	×	×	×
K3E	○	○	○
A4S	○	○	○
A4T	○	○	○
A4V	Weak	○	○
V5L	Weak	○	○
C6F	○	○	○
C6G	○	○	○
C6Y	○	○	○
V7E	Weak	Weak	○
L8Q	×	○	○
L8V	×	○	○
G10R	×	○	○
G10V	×	○	○
G12R	×	○	○
V14G	○	○	○
V14M	×	○	○
G16A	○	○	○
G16S	○	○	○
N19S	Weak	Weak	○
F20C	○	○	○
E21G	Weak	Weak	○
E21K	Weak	Weak	○
Q22L	○	○	○
V29A	○	○	○
G37R	○	×	○
L38R	○	×	○
L38V	○	×	○
E40G	Weak	×	○
G41D	○	○	○
G41S	○	○	○
H43R	○	○	○
F45C	○	○	○
H46R	○	○	○
V47A	○	○	○
V47F	○	○	○

Mutants	MS785	MS27	Mix
H48Q	○	○	○
H48R	○	○	○
E49K	Weak	Weak	○
T54R	×	×	×
C57R	○	○	○
S59I	Weak	Weak	○
N65S	○	○	○
P66A	○	○	○
L67R	×	×	×
G72C	○	○	○
G72S	○	○	○
D76V	○	○	○
D76Y	○	○	○
H80R	○	○	○
L84F	○	○	○
L84V	○	○	○
G85R	○	○	○
G85S	○	○	○
N86D	Weak	○	○
N86I	○	○	○
N86K	○	○	○
N86S	○	○	○
V87A	○	○	○
V87M	○	○	○
T88Δ	○	○	○
A89T	○	○	○
A89V	○	○	○
D90A	○	Weak	○
D90V	○	○	○
G93C	○	○	○
G93D	○	○	○
G93R	○	○	○
G93S	○	○	○
G93V	○	○	○
G93A	○	○	○
A95T	○	○	○

*Yellow : MS785 epitope

Green : MS27 epitope

Mutants	MS785	MS27	Mix
A95V	○	○	○
D96N	×	×	×
D96V	×	×	×
V97L	○	○	○
V97M	○	○	○
I99V	○	○	○
E100G	○	○	○
E100K	○	○	○
D101G	○	○	○
D101H	○	○	○
D101N	○	○	○
D101Y	○	○	○
I104F	○	○	○
S105L	Weak	○	○
S105Δ	○	○	○
L106V	○	○	○
G108V	○	○	○
C111Y	○	○	○
I112M	○	○	○
I112T	○	○	○
I113F	○	○	○
I113T	○	○	○
G114A	○	○	○
R115G	○	○	○
T116R	○	Weak	○
L117V	○	○	○
V118L	○	○	○
V118+	○	○	○
D124G	○	○	○

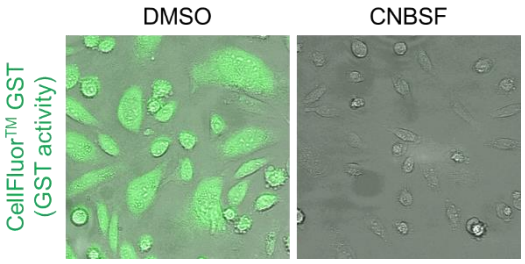
Mutants	MS785	MS27	Mix
D124V	○	○	○
D125H	○	○	○
L126S	○	○	○
L126*	○	Weak	○
L126Δ	○	○	○
G127+	○	○	○
E132+	○	Weak	○
E133V	○	○	○
E133Δ	○	○	○
S134N	○	○	○
N139D	○	○	○
N139H	○	○	○
N139K	○	○	○
A140G	○	○	○
G141E	○	○	○
G141*	○	○	○
L144F	○	○	○
L144S	○	○	○
A145G	○	○	○
A145T	○	○	○
C146R	○	○	○
C146*	○	○	○
G147D	○	○	○
G147R	○	○	○
V148G	○	○	○
V148I	×	×	×
I149T	○	○	○
I151S	○	Weak	○
I151T	○	Weak	○

Related Product

CellFluor™ GST <Cell-based GST Activity Assay Reagent >

CellFluor™ GST is a novel fluorescent probe for monitoring wide GST members' activity both *in celluo* or *in vitro*. CellFluor™ GST releases green fluorophore rhodamine 110 upon GST activities. This probe has cell-permeability and can detect intracellular GST activity.

Catalog No. FDV-0030
Size 0.1 µmol
Features
- Easy and quick protocol
- Broad specificity for various GST family members
- Ex/Em: 496 nm/520 nm
  (Compatible with commercial FITC filters)



Zin-Pro Capture < Mobile Zinc-Responsive Protein Labeling Reagent >

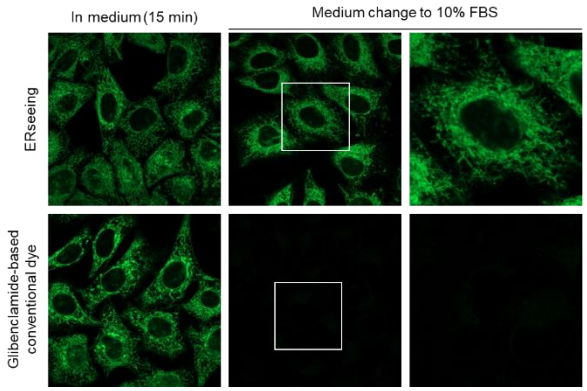
Mobile Zn^{2+}, the second class of zinc ion, is free form or loosely bound to protein surface and plays important roles in intracellular zinc homeostasis. Zin-Pro Capture is a mobile Zn^{2+} ion-responsive protein labeling reagent which labels fluorescein tag to proximal proteins of mobile Zn^{2+} ion and enables to analyze zinc homeostasis in protein basis.

Catalog No. FDV-0013A/B
Size 25 µg or 3x 25 µg
Features
- Label proximal proteins of mobile Zn^{2+} ion
- Labeled proteins by this reagent can be purified by immunoprecipitation with anti-fluorescein antibody
- Enable to identify mobile Zn2+-associated proteins by WB or MS analysis after purification

ERseeing <Endoplasmic reticulum Green>

ERseeing is a novel type of ER-staining dye and shows little pharmacological effects compared with conventional glibenclamide-based ER dyes. ERseeing is irreversible staining and is compatible with medium change for long-term imaging.

Catalog No. FDV-0038
Size 10 nmol
Features
- Recommended Ex/Em: 509 nm/524 nm
- Lless pharmacological effect on ER proteins
- Suitable for long-term live cell imaging



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