Version 03-06/24

User's Manual

Total ATG13 ELISA Kit

REF **DEIASL623**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Total ATG13 ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Atg13 protein.

General Description

Autophagy is a catabolic process for the autophagosomic-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with a number of physiological processes including development, differentiation, neurodegeneration, infection, and cancer (3). The molecular machinery of autophagy was largely discovered in yeast and referred to as autophagy-related (Atg) genes.

Atg13/Apg13 was originally identified in yeast as a constitutively expressed protein that was genetically linked to Atg1/Apg1, a protein kinase required for autophagy (4). Overexpression of Atg1 suppresses the defects in autophagy observed in Atg13 mutants (4). Autophagy requires a direct association between Atg1 and Atg13, and is inhibited by TOR-dependent phosphorylation of Atg13 under high-nutrient conditions (5). Similarly, mammalian Atg13 forms a complex with the Atg1 homologues ULK1/2, along with FIP200, which localizes to autophagic isolation membranes and regulates autophagosome biogenesis (6-8). mTOR phosphorylates both Atg13 and ULK1, suppressing ULK1 kinase activity and autophagy (7-9). ULK1 can directly phosphorylate Atg13 at a yet unidentified site, presumably to promote autophagy (7,8). Additional studies suggest that Atg13 and FIP200 can function independently of ULK1 and ULK2 to induce autophagy through an unknown mechanism (10).

ULK1-dependent phosphorylation of Atg13 at Ser355, which corresponds to Ser318 of isoform 2 of Atg13, leads to the recruitment of Atg13 to damaged mitochondria, enabling efficient mitophagy (11).

Principles of Testing

Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with Atg13 protein in a single step. The plate is then extensively washed and TMB reagent is added for signal development. The magnitude of absorbance for the developed color is proportional to the quantity of Atg13 protein.

*Antibodies in this kit are custom formulations specific to kit.

Reagents And Materials Provided

1. Atg13 Rabbit mAb Coated Microwells*: 96 tests. 4°C
2. Atg13 Rabbit Detection mAb: 1 each Red (Lyophilized). 4°C
3. HRP Diluent: 5.5 ml. Red. 4°C
4. TMB Substrate: 11 ml. 4°C
5. STOP Solution: 11 ml. 4°C
6. Sealing Tape: 2 each. 4°C

7. ELISA Wash Buffer (20X): 25 ml. 4°C
8. Cell Lysis Buffer (10X): 15 ml. -20°C

*12 8-well modules – Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

Specimen Collection And Preparation

Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 mL ice-cold 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at −80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/mL. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5-10 mL ice-cold 1X PBS.
3. Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at −80°C in single-use aliquots.

Reagent Preparation

NOTE: Prepare solutions with deionized/purified water or equivalent.

1. **Microwell strips**: Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
2. **Detection Antibody**: Reconstitute lyophilized Detection Antibody (red colored cake) with 5.5 mL HRP Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted Detection Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.
3. **HRP Diluent**: Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to

HRP.

4. **1X ELISA Wash Buffer**: Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
5. **1X Cell Lysis Buffer**: Prepare by diluting 10X Cell Lysis Buffer to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: When using to prepare cell lysates, add Protease/Phosphatase Inhibitor Cocktail (#CD5872, not supplied) and 1 mM phenylmethyl-sulfonyl fluoride (PMSF, #CD8553, not supplied) immediately before use.
6. **TMB Substrate**: Bring to room temperature before use.
7. **STOP Solution**: Bring to room temperature before use.

Assay Procedure

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Reagent Preparation).
2. Samples should be undiluted or diluted with 1X Cell Lysis Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the Detection Antibody. Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical results across a range of lysate concentration points.
3. Add 50 µL of each sample to the appropriate wells.
4. Add 50 µL of the Detection Antibody to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
6. Gently remove the tape and wash wells:

a. Discard plate contents into a receptacle.

b. Wash 4 times with 1X Wash Buffer, 200 µL each time for each well.

c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.

d. Clean the underside of all wells with a lint-free tissue.

7. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
8. Add 100 µL of STOP Solution to each well. Shake gently for a few seconds. NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.
9. Read results:

a. **Visual Determination**: Read within 30 min after adding STOP Solution.

b. **Spectrophotometric Determination**: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

Sensitivity

The Total ATG13 ELISA Kit detects endogenous levels of Atg13 protein. The kit sensitivity is shown in Figure

1. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

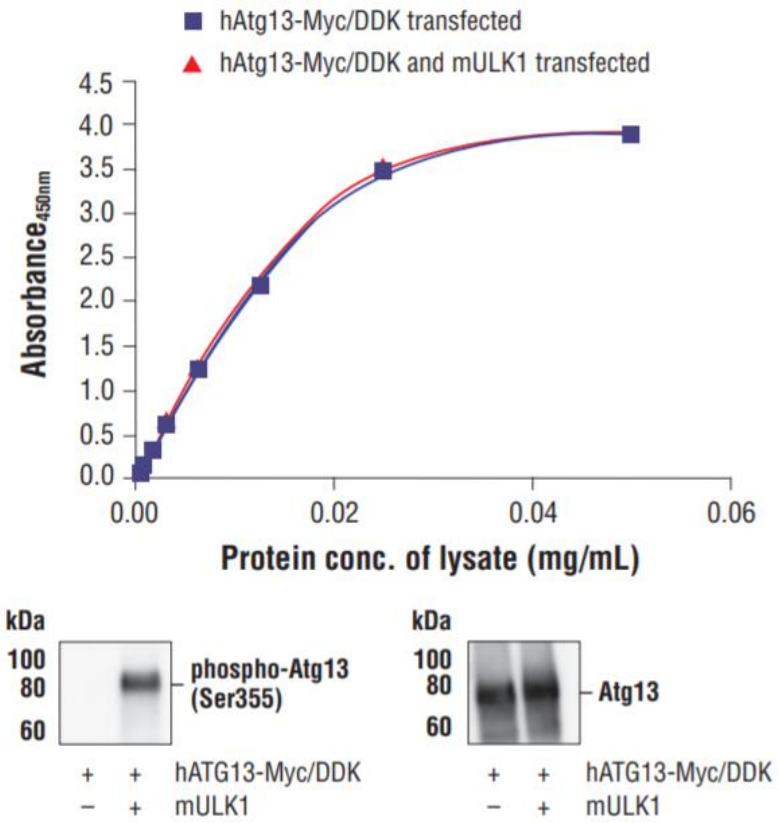

Figure 1. Co-transfection of 293T cells with constructs expressing human Atg13 and mouse ULK1 increases phosphorylation of Atg13 at Ser355 compared to cells transfected with only an Atg13 expression construct, but does not affect the level of total Atg13. The relationship between lysate protein concentration from Atg13/ULK1 co-transfected and Atg13-only transfected cells and the absorbance at 450 nm using the Total ATG13 ELISA Kit is shown in the upper figure. The corresponding western blots using phospho-Atg13 (Ser355) antibody (left panel) and Atg13 antibody (right panel) are shown in the lower figure. 293T cells were either co-transfected with constructs expressing Myc/ DDK-tagged full-length human Atg13 protein (hAtg13-Myc/DDK) and mouse ULK1 (mULK1) or just transfected with a construct expressing Myc/DDK-tagged full-length human Atg13 protein (hAtg13-Myc/DDK), and then lysed.

References

(1) Reggiori, F. and Klionsky, D.J. (2002) Eukaryot Cell 1, 11-21.

(2) Codogno, P. and Meijer, A.J. (2005) Cell Death Differ 12 Suppzl 2, 1509-18.

(3) Levine, B. and Yuan, J. (2005) J Clin Invest 115, 2679-88.

(4) Funakoshi, T. et al. (1997) Gene 192, 207-13.

(5) Kamada, Y. et al. (2000) J Cell Biol 150, 1507-13.

(6) Ganley, I.G. et al. (2009) J Biol Chem 284, 12297-305.

(7) Hosokawa, N. et al. (2009) Mol Biol Cell 20, 1981-91.

(8) Jung, C.H. et al. (2009) Mol Biol Cell 20, 1992-2003.

(9) Kim, J. et al. (2011) Nat Cell Biol 13, 132-41.

(10) Alers, S. et al. (2011) Autophagy 7, 1423-33.