

Induction of autophagy in mammalian cells

Akiko Kuma, Noboru Mizushima

METHOD

1. Plate cells in DMEM so that they will be 70-80% confluent at the next day. Starvation response will be reduced when they reach high confluency.
2. Aspirate medium, wash the cells one time with 1XDPBS of the equivalent volume of medium (when the cells are plated on slide glasses, wash twice).
3. Culture the cells in starvation medium (see below) in 5% CO₂ incubator to induce autophagy. For many cell lines (e.g. HeLa, MEF, HEK293 cells), 1-2 h starvation is enough to induce autophagy at maximum level in amino acid and serum-starvation (–AA–serum) medium.

MEDIUM

- AA and serum-starvation (–AA–serum) medium
D-MEM (High glucose) with Sodium Pyruvate, without Amino Acids (Wako, cat. no. 048-33575).
- AA-starvation (–AA) medium
Add 10 % dialyzed serum into –AA–serum medium.
- Dialyzed serum
To remove amino acids from FBS, FBS is dialyzed with Slide-A-Lyzer Dialysis Cassettes according to manufacturer's protocol. After dialysis, filtrate the serum using Millex-HV filter (0.45 µm). Keep at -4°C.
- DMEM
Dulbecco's modified eagle's medium (DMEM), high glucose (Sigma-Aldrich, cat. no. D6546) with 10% FBS, 2 mM L-glutamine and penicillin-streptomycin.

MATERIALS AND REAGENTS

- Slide-A-Lyzer Dialysis Cassettes 3,500 MWCO 30 ml capacity (Thermo Scientific, cat. no. 66130)
- Dulbecco's phosphate-buffered saline (DPBS, 10×) (Life technologies, cat. no. 14200-075)
- L-glutamine, 200 mM solution (Life technologies, cat. no. 25030-081)

- Penicillin-streptomycin, liquid (Life technologies, cat. no. 15070-063)

TROUBLESHOOTING TIPS

- Sometimes autophagy is not induced well when the cells are plated on slide glasses. To remove the left over medium between glass slide and bottom of the dish, it is advisable to lift glass slide up and down using tweezers, while glass slide is still submerged in PBS. Repeat washing with PBS one more time.
- Culturing in –AA–serum medium induces autophagy in 1-2 h in many cell types, on the other hand, sensitivity for serum starvation is depend on cell types. Glucose starvation is usually not effective to induce autophagy.