

U2OS TDP-25-TGFP

Experimental Assay



1. Introduction

- The TDP-25-tGFP U2OS cell line is a powerful tool for investigating the mechanisms underlying neurodegenerative diseases like Amyotrophic lateral sclerosis (ALS) or Frontotemporal dementia (FTD).



2. Product components and recommended storage conditions

- U2OS TDP-25-tGFP (Innoprot P30723)
- 1 vial 3×10^6 cells in Freezing Media
- Storage: Immediately upon receipt, storage in liquid nitrogen



3. Biological Activity

- This cell line has been validated for cellular response to stimulation with sodium arsenite (Sigma-Aldrich S7400).
- Mycoplasma testing: The cell line has been screened using a Mycoplasma PCR Detection Kit (Abm G238) following manufacturer's instructions.



4. Recommended Reagents to Be Supplied by the User

- DMEM/Nutrient Mixture F-12 Ham (Sigma-Aldrich D8437)
- Fetal bovine serum (FBS) (Sigma-Aldrich F7524)
- DPBS with calcium and magnesium (Sigma Aldrich D8662)
- Opti-MEM (ThermoScientific 31985-047)
- Greiner CELLSTAR® 96 well plates flat bottom black polystyrene wells (with micro-clear bottom) (Greiner M0562-32EA)
- Hoechst 33342 (ThermoFisher H1399)



5. Recommended Equipments

- Class II biological safety cabinet
- Hemacytometer / Cell counter
- Incubator humidified 37°C, 5% CO₂
- Inverted microscope
- Image analysis: Appropriate filter for the turboGFP protein fluorescent signal, with excitation and emission peaks at 482 nm and 502 nm, respectively.



6. Image Assay: Experimental Protocol

- Day 1. Thaw the cells (3x10⁶ cells per T25).
- Day 2. Maintain cells in DMEM-F12 supplemented with 10% FBS at 37 °C in a humidified 5% CO₂ atmosphere.
- Day 3. Plate cells at a concentration of 8,000 cells/plate in a 96-well plate and maintain them in DMEM-F12 medium supplemented with 10% FBS during 24h at 37 °C in a humidified 5% CO₂.
- Day 4: Treat cells with 50 µM sodium arsenite diluted in OptiMEM and incubate overnight. Add Hoechst at a final concentration of 10-20 µg/ml directly to the wells without removing the media and incubate for 20–30 minutes at 37 °C, 5% CO₂.
- U2OS TDP-25-tGFP signaling can be quantified analyzing spot number by image analysis.