Product description

Murine embryonic stem cells with an inducible and reversible (via degron AID) KO of Mettl3 - protein used for m6A methylation of RNA

Name: METTL13-AID cell line

Research fields: Stem cell biology

Parental cell: E14 murine ES cells (129Ola strain)

Organism: Mouse

Gender: Male

Tissue: Blastocyst

Growth properties: Adherent

Model: Genetically modified cell line; Knock in

Model description: The cell line contains OsTIR1 at the TIGRE locus. The AID tag is inserted in the N terminus of the Mettl14 gene.

CRISPR edited: Yes

Conditional description: Depletion is achieved upon Auxin treatment (degradation of the AID-tagged proteins).

Contributor(s)

Inventors: Déborah Bourc'his, Tomasz Chelmicki
Institute: Institut Curie

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Recommended medium: 2i + LIF made of 50% Neurobasal™ Medium (Gibco™), 50% DMEM/F12, 2 mM L-glutamine, 0.1 mM β-mercaptoethanol, N-2 supplement (Gibco™), B-27™ medium supplement (Gibco™), 1,000 U/mL Leukemia Inhibitory Factor (Mouse LIF), 4.5 µM GSK3 inhibitor (CT99021), 1.5 µM MEK inhibitor (PD0325901).

Cultureware: 0.2% gelatin-coated flasks

Subculture: Accutase™ (1mL for a T25, 3 minutes at 37 °C). After 3 minutes, resuspend in 4mL of fresh 2i media, centrifuged, remove the supernatant and resuspend in fresh complete medium (3-4 mL). Seed 250,000-

500,000 in a T25 flask (cells will have to be split in 2 days or 4 depending on seeding density) and change media the next day.

Cryopreservation: 2X freezing medium made of 40% KnockOut™ Serum Replacement (Gibco™, ref. 10828-028), 40% 2i+ LIF and 20% DMSO. Freeze at a density of 2 - 4 million cells per mL. After counting, resuspend the cells in a specific volume of 2i+ LIF medium and add the same volume of cold 2X freezing media. Mix and distribute the cells across the vials before freezing gradually. Store long term at -150 °C or vapor phase of liquid nitrogen.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2.

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the cell suspension into a 15 mL sterile conical tube containing 4 mL of prewarmed 2i+ LIF media. Centrifuge the total volume (4 mL of media+ 1 mL of cell suspension) for **5 minutes (1200 rpm or 290 g)**.
5. Aspirate the supernatant and resuspend the pellet in 5 mL fresh 2I+ LIF medium and transfer to a T25 flask.

References

- Tomasz Chelmicki et. al. 2021 Nature. 591(7849):312-316. PMID: 33442060

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner:
METTL13-AID cell line, was invented at Institut Curie (CancerTools.org #162154).