

Product Sheet

H_RANKL U2OS Cell Line

Catalog number: GM-C30776

Version 3.3.1.260805

Description	H_RANKL U2OS Cell Line is a clonal stable U2OS cell line that constitutively expresses the human RANKL gene, constructed using lentiviral technology.
Quantity	5E6 Cells per vial, 1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Target	Human RANKL
Gene ID/Uniprot ID	O14788-1
Host Cell	U2OS
Recovery Medium	McCoy's 5A+10% FBS+1% P.S
Growth medium	McCoy's 5A+10% FBS+1% P.S+0.5 µg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO ₂
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
McCoy's 5A	VivaCell/C3020-0500
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Puromycin	Genomeditech/GM-040401
ABflo® 647 Rabbit anti-Human CD254/RANKL mAb	abclonal/A26761

Figures

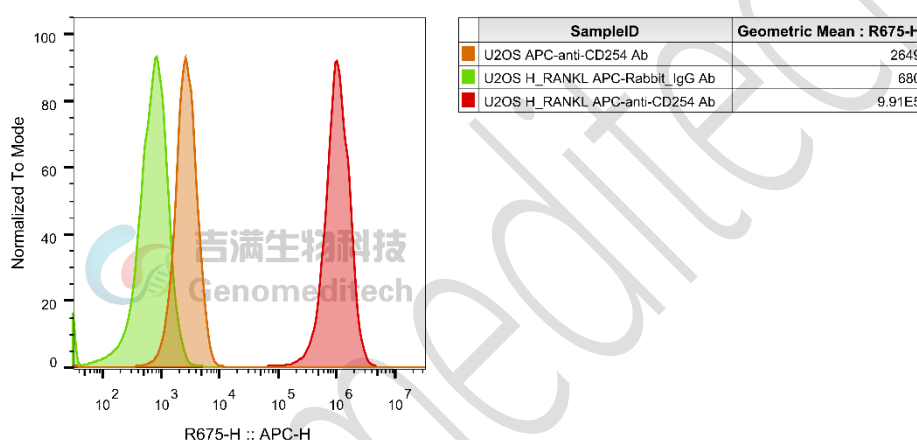


Figure 1 | H_RANKL U2OS Cell Line (Cat. GM-C30776) was determined by flow cytometry using ABflo® 647 Rabbit anti-Human CD254/RANKL mAb (abclonal/A26761).

Cell Recovery

Recovery Medium: McCoy's 5A+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

- Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
- Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately 176 x g for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.

- e) Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- Centrifuge at 176 x g for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 4E6 cells/mL.
- Aliquot 1 mL into each vial.
- Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: McCoy's 5A+10% FBS+1% P.S+0.5 µg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 3 minutes at 37°C).
- Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

Notes

- It is normal to observe a higher number of dead cells immediately after thawing. The condition will improve significantly after adjustment. Once the cells stabilize, the number of dead cells will decrease after subculturing, and the cell growth rate will become stable.

Sequence

RANKL O14788-1

MRRASRDYTKYLRGSEEMGGGPGAPHEGPLHAPPPAPHQPPAASRSMFVALLGLGLGQVVCVSVALFFYFR
AQMDPNRISEDGTHCIYRILRLHENADFQDTTLESQDTKLIPDSCRRIKQAFQGA VQKELQHIVGSQHIRAEKA
MVDGSWDLAKRSKLEAQPFAHLTINATDIPSGSHK VSLSSWYHDRGWAKISNMTFSNGKLIVNQDGFYYL

YANICFRHHETSGDLATEYLQLMVYVTKTSIKIPSSHTLMKGGSTKYWSGNSEFHFYSINVGGFFKLRSGEIS
IEVSNPSLLDPDQDATYFGAFKVRDID

Related Products

RANK:RANKL	
RANK Reporter 293 Cell Line	
Anti-H_RANKL hIgG2 Antibody(Denosumab)	

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