Thawing of cells (HA-RSPo1-Fc; 3B)

1. This cell line is stable transfectant of 293T cells expressing mouse R-spondin1 protein tagged with C-terminus HA and N-terminus Fc. This was selected clone (3B) by zeocin.
2. 1~3x10^6 cells in 1 ml of FCS supplemented with 10% DMSO.
3. Thaw the tube in warm water quickly.
4. Centrifuge at 700rpm for 5min with 40~50 mL of PBS containing 5~10% FCS.
5. Discard the supernatant and resuspend the cells in the complete medium of DMEM high glucose (GIBCO ##11965-092) supplemented with 10% FCS and PSG (GIBCO #10378-016).
6. Take them into a T25 cell culture flask.
7. The day after, the cells should become confluent.
8. You can select the transformants again by zeocin (300μg/ml, Invitrogen, #250-01) resistance, but you don't need to add zeocin in media normally.

Purified the protein (HA-RSpo1-Fc)

1. After the cells become 90~95% confluent, the cells are cultured with CD 293 medium (GIBCO, #11913-019) with L-glutamine.
2. After 7~14 days culture, the supernatant is collected (and stored -80°c).
3. The protein is purified by Protein A Agarose Purification Kit (KPL, #553-50-00).

Updated Dec 16, 2009

Akifumi Ootani, MD, PhD.
aootani@gmail.com

Purification of HA-R-spondin1-Fc protein

1. The stable 293T cell line expressed HA-RSpo1-Fc (clone 3B) will be cultured and passaged in DMEM high glucose (GIBCO #11965-092) supplemented with 10% FCS and PSG (GIBCO #10378-016).
2. Maintain the confluent cells with CD293 (GIBCO #11913-019) containing L-glutamine (GIBCO #25030) for 7~14 days without medium change.
   • Triple flask (Nunc #132913, http://nuncbrand.com) with 150-200ml of media.
3. Correct the supernatant and spin it (3000rpm, 15min). <<On ice or at 4°C>>
4. Filtrate (0.22μm, Corning #430517) the SNT. <<On ice or at 4°C>>
5. Purify the protein using Fc tag with KPL Protein A Agarose Purification Kit (KPL #553-50-00) as manufacturer's instruction. <<at 4°C>>
   • From 1L of SNT, you can get around 2mg of RSpo1 protein.
6. Dialyze by 1L of PBS with 10% Glycerol (>4h) x2 using Slide-A-Lyzer 10K Dialysis cassettes (Thermo #66380, http://www.piercenet.com). <<at 4°C>>
7. Aliquot the purified protein to avoid repeated freezing/thawing cycles.
8. Freeze it with liquid nitrogen (snap frozen) and store at -80°C.
9. Check the purified protein by both Coomassie and Bradford protein assay.
10. Good luck!

2009.12.16. updated

Akifumi Ootani, MD, PhD.