R10 media

RPMI + 10% FBS + supplements

500 ml (1 bottle)	RPMI1640 with L-Glu (Mediatech)
55 ml	Heat-inactivated FBS (PAA)
5.5 ml	L-Glutamine (200 mM) (Mediatech)
5.5 ml or 11 ml*	Penicillin/Streptomycin (100x or 50x) (Mediatech)
5.5 ml	HEPES buffer (1 M) (Mediatech)

1. Add the ingredients listed above to the bottle of RPMI and mix well by shaking the bottle.
2. Store at 4 C.

Notes

- FBS should be heat inactivated (HI) at 56 C for 30 min if it has not already been heat inactivated by the supplier.
- L-glutamine is provided in a 200 mM stock and diluted 1:100 to give a 2 mM working concentration
- Penicillin / streptomycin is provided as either a 100x or 50x solution. In the end, we want a working concentration of 100 U/ml / 100 ug/ml.
    - 100x solution (10,000 U/ml / 10,000 ug/ml stock): Add 5.5 ml to dilute 1:100.
    - 50x solution (5,000 U/ml / 5,000 ug/ml stock): Add 11 ml to dilute 1:50.
- HEPES buffer is provided in a 1 M stock and diluted 1:100 to give a 10 mM working concentration.

IL-2 stock solution

Recombinant human IL-2 at 10^6 U/ml

1. Make 10 ml of 100 mM acetic acid in autoclaved Nanopure water.
    a. For example, start with glacial acetic acid (17.5 M) and dilute several times in water.
2. Make 10 ml of 0.2% BSA in PBS.
3. Sterile filter each solution separately using a 0.2 um syringe filter.
4. Take a vial of recombinant human IL-2 (10 ug, Peprotech, Cat. #200-02). Do a hard spin in the centrifuge to pellet any lyophilized protein flakes that might be sticking to the wall or lid.
5. Resuspend the IL-2 in 50 ul of the sterile-filtered 100 mM acetic acid solution.
6. Add 50 ul of the 0.2% BSA solution.
7. Mix by pipetting.
8. Divide the stock solution (100 ul of IL-2 at 10^6 U/ml) into 5.5 ul aliquots.
9. Store the aliquots at -80 C.

Notes

- According to the Peprotech website, the IL-2 has a specific activity of $>10^7$ U/mg, so a 0.1 mg/ml stock should correspond to at least 10^6 U/ml.

R10/100 media

R10 + 100 U/ml IL-2

50 ml	R10 media
5 ul	IL-2 stock solution (10^6 U/ml), freshly thawed

1. Add 50 ml R10 to a 50 ml conical tube.
2. Take an aliquot of IL-2 stock solution from the -80 C freezer and thaw on ice.
3. Add 5 ul of IL-2 to the R10 and mix well.
4. Use immediately or store at 4 C for up to 1 week.

Notes

- The IL-2 in the R10/100 is only good for 1 week, so make R10/100 in small batches (e.g., 50 ml) and only use for one or two feedings.

NK-92 cell culture

Background

- NK-92 is an IL-2 dependent NK cell line derived from a patient with lymphoma.
- NK-92 are suspension cells that grow as clumps and single cells.
- It is common for cell debris to be visible in the culture media.
- For more information, see the ATCC website (Cat. # ATCC CRL-2407)
    - http://www.atcc.org/products/all/CRL-2407.aspx#357C3571006A4259B64650D34DF19048
    - The culture media recommended on the website is different from the one described in this protocol.

Thawing cells

1. Pre-warm (37 C) 9 ml of R10 media in a 15 ml conical tube.
2. In a separate 15 ml conical tube, pre-warm (37 C) 10 ml of R10/100 for culturing.
3. Retrieve the vial of cells from the LN2 storage.
4. Rapidly thaw the cells in a 37 C water bath by gently shaking the vial back and forth. Stop when a small sliver of ice remains (typically ~1 min).
5. Bring the cells to the TC hood and wipe the vial down with 70% ethanol.
6. Use a P1000 pipette to transfer ~500 ul of the warmed R10 media to the thawed cells. Transfer drop-by-drop to avoid osmotic shock.
7. Transfer the thawed cell suspension to the tube of R10.
8. Centrifuge at 340 g (1300 rpm) for 7 min to pellet the cells.
9. Aspirate media and resuspend cells in the 10 ml of pre-warmed R10/100 media.
10. Count cells.
11. Transfer to a T25 culture flask and place in the incubator (37 C, 5% CO2). The flask can be horizontal or vertical; either is fine.

Culturing cells

NK-92 cells are sensitive to overgrowth and media exhaustion, so they should be split every 2-3 days. They should be maintained at 0.1 – 0.4 x 10^6 cells/ml in R10/100.

1. Pre-warm (37 C) and equilibrate R10/100 media by placing the media in the incubator in a tube with the lid loosened or in a cell culture flask.
2. Move the cell suspension to a conical tube. Pipette gently to break up the clumps and form a single cell suspension.
3. Count cells.
4. Centrifuge at 340 g (1300 rpm) for 7 min to pellet the cells.
5. Resuspend cells in an appropriate amount of R10/100 and move to a culture flask. Place in the incubator.

Freezing cells

1. Collect cells into a conical tube and count cells.
2. Prepare freezing media. Keep on ice until use.

Fetal bovine serum (FBS), heat inactivated	90% by volume (e.g., 9 ml)
DMSO	10% by volume (e.g., 1 ml)

3. Centrifuge cell suspension at 340 g (1300 rpm) for 7 min to pellet cells.
4. While cells are spinning down, label the appropriate number of cryovials.
5. Resuspend cells at desired concentration (e.g., 10^7 cells/ml) in ice-cold freezing media.
6. Aliquot 0.5 – 1 ml of cell suspension (5 – 10 M cells/vial) into the cryovials.
7. Place the cryovials in a chilled Mr. Frosty (filled with isopropyl alcohol).
8. Transfer Mr. Frosty to a -80 C freezer.
9. The next day, remove the cells from Mr. Frosty and transfer them to a box in the liquid nitrogen storage vessel (-196 C).