

Media Preparation

Notes on location/aliquots of reagents:

- We typically pool the Pen/Strep and L-Glutamine together into a 15 mL conical and freeze down in the -20°C with the label "P/L" located in the B3 lab (see below)
- Serum (bovine and human) is found in the -80°C. When only 2-3 aliquots remain, remove a 500ml container from the -80°C and place in the 4°C to thaw. We typically aliquot between 50-55mL of FCS in 50 mL conicals and kept in the 4°C, TC lab.
- Brefeldin A is located in the -20°C in the B3 lab

Notes on making solutions

As all solutions listed can/will be used for cell culture, sterile technique **must** be used. Media preparation should be done in a hood and media containing FCS should be filtered through a 0.22µm filter system (a 500mL filter system can be used). New media should be labeled with preparer's initials, date, and what it contains.

R+

RPMI 1640 medium (Sigma, St. Louis, Mo)	-500ml bottle
Penicillin/streptomycin(FC 50U/ml)	-5ml

R-10 (10% FCS)

RPMI 1640 medium (Sigma, St. Louis, Mo)	-500ml bottle
10 % FCS (heat inactivated-1 hour, 56 °C)	-55ml
L-glutamine (fc 2 mM)	-5ml
Penicillin/streptomycin (FC 50U/ml)	-5ml
HEPES (fc 10mM)	-5ml
(N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid)	

R 20 (20% FCS)

RPMI 1640 medium	-500ml
20% FCS (heat inactivated)	-125ml
L-glutamine	-5ml
Penicillin/streptomycin (FC 50U/ml)	-5ml
HEPES 10mM	-6.2ml

R 20++ (20% FCS)

Add to 46.75ml R20 medium in 50ml conical:	
Sodium Pyruvate	-0.5ml
Non-essential amino acid solution (NEAA)	-0.5ml
Apo-Transferrin (1mg/ml stock)	-2ml
2-mercaptoethanol (2-ME)	-250ul

RAB-10 (10% Human AB serum)

RPMI 1640 medium (Sigma, St. Louis, Mo)	-500ml bottle
Human AB Serum (GemCell)	-55ml
L-Glutamine (fc 2 mM)	-5ml
Penicillin/Streptomycin(FC 50U/ml)	-5ml
HEPES 10mM	-5ml

RAB-10 (10% Human AB serum) + Fungizone

RPMI 1640 medium (Sigma, St. Louis, Mo)	-500ml bottle
Human AB Serum (GemCell)	-55ml
L-Glutamine (fc 2 mM)	-5ml
Penicillin/Streptomycin(FC 50U/ml)	-5ml
HEPES 10mM	-5ml
Fungizone (FC 2.5ug/ml) (Gibco)	-5ml

Penicillin/Streptomycin + L-glutamine pops

Thermo Scientific™ HyClone™ Penicillin-Streptomycin Solution (Fisher)	-100ml
Thermo Scientific™ HyClone™ L-Glutamine (Fisher)	-100ml

Thaw one 100ml container of each in the water. Thoroughly mix equal volumes of the above reagents and aliquot 10ml into 15ml Falcon conicals. Label the aliquots with “P/L” and put your initials and date on each conical. Place in the -20°C, TC lab.

Brefeldin A

We order BFA in 5mg vials (Sigma #B7651). Reconstitute the BFA with 1mL DMSO (5mg/ml) and then create 5 working solutions by aliquotting 200µl of the resuspended BFA into separate 1.7mL screw cap tubes and adding 800µl of PBS bring the final volume up to 1mL (final concentration = 1mg/mL). Label the tube “BFA 1mg/mL” and the date.

R10/IL-2 medium

Make R10 as outlined above, then add IL-2 at 50U/ml. Use an entire aliquot of IL-2 from the -80° C freezer. The aliquots are made from the Roche stock, which is at 10⁵ U/ml. The same applies if making RAB-10/IL-2 medium. Often, it is better to just make a 50ml conical aliquot, so that you can insure that the IL-2 and media is fresh, and you don't have to make an entire 500ml bottle to use in a limited time.

Wipe bottles with alcohol after removing them from the water bath. Do not use media directly from the bottle (unless you know you are going to use it up the same day)-- this will give you an extra layer of protection against unexpected contamination. Pour some media into a conical instead and pipet from there. Wipe all bottles with alcohol before putting them in the hood.

FCS

Thawed bottles of FCS need to be heat inactivated prior to aliquoting/use. Allow FCS to thaw 1-2 days at 4°C and place in water bath. Place a control bottle filled with water in the water bath and insert a thermometer into this control bottle. Set the water bath's temperature to 56°C and

once the thermometer in the control bottle reaches 56°C begin timing. After 30min, remove the FCS and set the water bath back to 37°C. Create 50ml aliquots of FCS in 50ml Falcon tubes. Label tubes with "FCS" and the date. Keep at 4°C