

Care of the R28 retinal precursor cell line

Gail M. Seigel, Ph.D.

Thawing cells: Place a vial of cells in 37° water bath until thawed. Slowly pipet the cells, under sterile conditions, into a 10 mm dish or a T75 flask in DMEM with 10% serum (see recipe below). As soon as the cells are attached, remove the medium and add fresh medium to the dish. This gets rid of the DMSO that was in the original freezing vial.

Ordinarily, every 2-3 days the cells either need to be fed or trypsinized. Trypsinization is accomplished as follows:

Rinse cell surface with 1 ml CMF-EDTA, pour off, then add 1 ml CMF-EDTA. Add 1 ml of 0.125% trypsin to the dish and incubate at 37°C for 3-5 min. Pipet the trypsin/CMF-EDTA solution over the cell sheet, mix well, and transfer a portion of the cell suspension to a fresh dish with DMEM+. I recommend a 1:2 or 1:3 split. If the cells get too sparse or too dense, they tend to die. However, there is a rather large window of acceptable cell density.

I would recommend scaling up the cell culture and freezing down aliquots for future use. For freezing, we centrifuge cells at low speed, resuspend the cell pellet in 900 µl of DMEM+, and add 100 µl of Dimethyl Sulfoxide. Prefreeze at -70°C overnight, then transfer to liquid Nitrogen.

R28 cell description:

R28 is an adherent retinal precursor cell line derived from postnatal day 6 Sprague-Dawley rat retina immortalized with the 12S E1A gene of adenovirus. The 12S E1A gene was introduced via an incompetent retroviral vector; therefore, no infectious virus is produced by R28 cells. The cells have been passaged 200 times thus far, and show no signs of senescence.

Recipes

DMEM+

420 mls DMEM D5523 incomplete (Sigma)
15 mls sodium bicarbonate (7.5% stock solution, w/v) (Sigma S5761)
50 mls calf serum (Hyclone, A2111-L-HI)
5 mls MEM non-essential amino acids (GIBCO, 11140-019)
5 mls MEM vitamins (GIBCO, 11120-029)
5 mls L-glutamine (200 mM stock) (GIBCO, 25030-024)
0.625 mls Gentamicin (stock is 80 mg/ml)

Important: Do not use the anti-fungal "Fungizone" (Amphotericin B) for R28 cell media. It is toxic to the cells.

CMF-EDTA:

Make CMF first:

NaCl	8.77 g
Na ₂ HPO ₄ ·7H ₂ O	2.28 g
KCl	0.2 g
KH ₂ PO ₄	0.2 g
NaHCO ₃	2.18 g

Dissolve these ingredients in 900 ml dH₂O. pH solution to 7.4. Then, bring up the volume to one liter with dH₂O. (Optional: add several drops of 0.2% phenol red to get the desired chablis color). Filter sterilize or autoclave.

CMF-EDTA: for every 100 mls of sterile CMF prepared above, add the following:

1 ml sterile 2% EDTA
1 ml sterile 10% glucose
125 ul gentamicin (final concentration is 50 ug/ml)