

## Cell Labeling with $^{35}\text{S}$ -methionine

### Materials:

glutamine

CS or FCS

$^{35}\text{S}$ -methionine- shelf of  $-80^\circ\text{C}$  freezer

DME (minus: methionine, glutamine, sodium pyruvate) Gibco

### Procedure:

1. To label one P100(P150), prepare the following mixture first, in 50ml Falcon, in hood:

- a. 1x final glutamine =  $35\lambda(90\lambda)$  of 100X per P100(P150)plate
- b. 1 mCi  $^{35}\text{S}$ . Wipe condensation and check wipe for radioactivity. Insert it in larger Epp. Put both in water bath. Spin down. OR, thaw in ice. Beware of radioactive vapors. Check fingers after opening cap.

**Don't get pipetman hot. Aliquot slowly. Technique: release tip while top trigger is still pressed down.**

Calculations

Assay date 5-13-93

Date of labeling procedure 6-21-94

39 days

Chart: 0.734 multiplication factor for decay.

Concentration on delivery date = 10 mCi/1.266ml

Conc \* decay factor = true concentration

$(10/1.266) * 0.734 =$

Desired amount of radioactivity/true concentration = volume of isotope needed for experiment.

(Est. 5% is lost during thawing)

c. 0.5% final FCS(if dialyzed can use more; 1% is fine) =  $18\lambda(45\lambda(0.5\%))$  or  $90\lambda(1\%)$  per P100(P150) plate (mix before using)

d. make total volume=3.5ml (9ml for P150) plus 0.5ml extra with met- DME. The extra is because you will lose some when you filter sterilize it.

**Use plastic pipets. Separate solid and liquid radioactive waste and dispose of appropriately. Cross out radioactive labels or signs before putting in trash. Avoid breathing fumes.**

2. For a short label, if all components are handled sterilely, you don't have to filter sterilize. For longer label, or, if you are concerned, filter sterilize with a 0.22um syringe filter and appropriate syringe.

3. Aspirate media.

4. Wash plate with met-DME 3X (3ml or 5ml for P100 or P150 respectively). Aspirate well after each wash.

5. Add labeling media. Add to corner of plate. Don't splash.

6. Plut plate in plexiglass box with activated charcoal in another open P100 plate. Wedge open labeling box lid for ~30min and then close top and open vent. Incubate at  $37^\circ\text{C}$  in 10%  $\text{CO}_2$  for 4.5-5.5 hrs.

Check work areas and yourself for radioactive contamination. Record results.