DIGE
LABELING

SOP TO PREPARE BUFFERS FOR DIGE LABELING AND 2D GELS.

SOLUTIONS

7.0 M urea/2M thiourea (50 ml)

1. Weigh 21 gm urea in a clean 150 ml glass beaker.
2. Weigh 7.6 gm thiourea and add to the urea.
3. Add enough MilliQ water to approximately 40-45 ml.
4. Add stir bar and mix on WARM stir plate until dissolved.
5. Transfer to a graduated cylinder and add MilliQ water to 50 ml.
6. Freeze in 1.5 ml aliquots (-20C) or de-ionize and freeze.

1M Tris, pH 8.5

1. Measure 80 ml mQ water into a 150 ml beaker.
2. Add12.11 gm of Tris into the water.
3. Titrate with concentrated HCL to pH 8.5.
4. Bring volume to 100 ml.
5. Store room temperature.

PROCEDURE FOR L-BUFFER

1. Thaw 7M urea/2M thiourea from -20C.
2. De-ionize over small MB-1 column (omit if previously de-ionized).
3. Add 40 mgm CHAPS per ml of de-ionized urea (4% CHAPS final concentration).
4. Add 30 ul 1M Tris, pH 8.5 per ml of buffer.
5. Store in 1 ml aliquots at -20C indefinitely.

PROCEDURE FOR R-BUFFER

To 1 ml of L-buffer add: 1 mg DTT

PROCEDURE FOR 2X-R-BUFFER

To 1 ml of L-buffer add: 2 mg DTT

PROCEDURE FOR PREPARING CyDyes

CyDyes purchased from GE Healthcare (Cat. No. 25-8010-65). This is a 5 nmol kit.

NOTE:

a) WORK IN SEMI-DARKNESS; DYES ARE SENSITIVE TO LIGHT.
b) USE HIGHEST QUALITY REAGENTS AVAILABLE.
c) STORE DMF WITH MOLECULAR SIEVE TO ABSORB FREE AMINES

Prepare Stock solution:

1. Dissolve dyes in 5 ul of fresh Dimethyl Formamide (DMF). Concentration is now 1 nmol/ul.
2. Store at -20C for 3 months.

Prepare Working solution:

1. Dilute 1 ul of dye with 4 ul of DMF (5 ul total; Concentration is now 200 pmol/ul).
2. Keep on ice during use.
3. Store at -20C for 3 weeks.

PROCEDURE FOR LABELING PROTEINS WITH CyDYEs

Note: All procedures performed in semi-darkness.

Flow chart:

A. Precipitate proteins.
B. Solubilize in DIGE labeling buffer (conc ~5-10 mg/ml).
C. Spin out insoluble material.
D. Use supernatant materials for labeling.
E. Label separately.
F. Combine.
G. IsoElectric Focusing.

PROCEDURE: Precipitation

1. Pipet 100 ugm of each protein (control and experimental) into separate 1.5 ml Eppie tubes. LABEL TUBES WELL. Perform precipitation on both samples.

NOTE: We are precipitating 100 ugm of protein, but predict that we will only resolubilize 75 ugm in this procedure.

2. Add mQ water so total volume = 100 ul; CHECK WITH LAB ASSISTANT.
3. Add 400 ul of MeOH; vortex.
4. Add 100 ul of Chloroform; vortex.
5. Add 300 ul MilliQ water; vortex.
6. Spin at 13,000 x g (top speed) in microfuge. RT, 1 minute.
7. Result: A layer at interface (= protein).
8. Remove and discard the upper layer from each tube.
9. Add 300 ul MeOH; vortex.
10. Spin at 13,000 x g, RT, microfuge, 2 minutes.
11. Proteins in pellet.
12. Remove supernatant materials.
13. Wash pellets one time with 100 ul of 100% MeOH.
14. Spin at 13,000 x g (top speed) in microfuge. RT, 1 minute.

15. Remove MeOH.
16. Air dry the pellets, 5 minutes, MAXIMUM.

PROCEDURE: Labeling

1. Resuspend pellets in 15 ul DIGE labeling buffer (L-buffer). KEEP SAMPLES SEPARATE.
2. 60 minutes, RT, Sonicate, vortex.
3. Add 0.2 ul 0.5 M NaOH. Mix by flicking. Spin in table top micro-cup fuge to collect liquid.
4. Test 0.3 ul of material on pH strip; ≥ pH 8.5? If not above pH 8.5, add 0.2 ul 0.5M NaOH. Flick, spin, test again.
5. Spin at 13,000 x g, RT, in microfuge, 10 minutes.
6. **Use supernatant fluids for all of the following procedures.**
7. Label two 0.5 ml Eppie tubes; Cy3, Cy5
8. Pipet 9 ul (~50 ugm) of appropriate protein into tubes Cy3 & Cy 5. **SEE INSTRUCTIONS GIVEN TO YOU BY THE LAB PROCTOR.**
9. **The Lab Proctor will take 4.5 ul (~25 ugm) of your proteins for POOLED Cy2 labeling.**
10. **Add 1 ul of appropriate dyes SEPARATELY to tubes Cy3 & Cy5; vortex briefly; spin briefly to collect liquid.**

NOTE: You will see that that the dyes used are inverted for some samples. This is intentional. This called 'dye-swap'. FOLLOW THE PROTOCOL GIVEN TO YOU. Don't worry about what your neighbor is doing.

11. React 60 minutes on ice, in the dark.
12. Add 1 ul 10 mM lysine to each reaction tube; vortex briefly; spin to collect liquid.
13. React 10 minutes on ice, in the dark.
14. The lab proctor will do the Cy2 labeling.

This labeling is done in the same fashion that you are labeling but at a larger scale. The sample (pooled internal standard) is composed of Control and Experimental proteins from each of you. This pooled sample is labeled with Cy2 and when

included in the gels, provides the analytical software with an internal reference (that contains ALL proteins) to permit the normalization need to determine changes in relative abundance and make-up for technical errors.

15. Combining labeled protein samples. **CAREFUL ATTENTION REQUIRED.**

16. Combine your Cy3 & Cy5-labeled proteins in one tube. Add 11 ul of the POOLED Cy2-labeled sample from the Lab Proctor and mix with your Cy3 & Cy5-labeled samples. (Total protein is 150 ugm.

17. Total volume = **33 ul IN ONE TUBE**.
18. Add 33 ul of 2X-R-buffer; Mix by pipetting.
19. Add 1.25 ul of pH 4-7 IPG buffer; mix. By pipetting
20. Add 182.8 ul 1X-R-buffer; Mix by pipetting and flicking. Spin briefly to collect liquids in micro-cup fuge.
21. Total volume = **250 ul IN ONE TUBE**.
22. Total protein ~150 ugm.
23. Spin materials 13,000 x g, 5 minutes, RT, to remove any particles.
24. Use this supernatant material for IEF, overnight.