IMMUNOFLORESCENCE PROTOCOL

This protocol is used for staining cells grown in 2-well glass chamber.

Reagents:

Fixation solution (make fresh in hood)		<u>0.1M Tris</u>
37% Formald.	4.3ml [4%]	
1xPBS	35.7ml	
Detergent soln. (150ml)		Blocking soln
Saporin	0.75g [0.5%]	10% FBS + 0.3% Triton-X in 1X PBS
Triton-100X	0.75ml [0.5%]	
1xPBS 150m	1	
Washing soln.(250ml)		Mounting (antifade) soln.
FBS	5ml [2%]	2.5% DABCO
Triton-100X	0.75ml [0.3%]	200mM Tris-HCl pH8.6
1xPBS 245m	l	90% glycerol

Procedure:

- 0. Clean coplin jars.
- 1. Pour out the media from the chambers and remove the plastic well.
- 2. Using the forceps, carefully remove the rubber linings.
- 3. Wash with 1xPBS in a coplin jar at RT for no more than <u>3min</u>.
- 4. Remove 1xPBS and add 40ml of Fixation soln. in the hood. Incubate at RT for 10min.
- 5. Fill a clean coplin jar with 0.1M Tris. Transfer the fixed slides to the 0.1M Tris and incubate at RT for 5min. ¹
- 6. Remove 0.1M Tris soln. and wash with 1xPBS for <u>3min</u>.
- 7. Remove 1xPBS and wash <u>3X 5min</u> in Detergent soln. at RT.
- 8. Fill a clean coplin jar with 1xPBS. Transfer the slides to the 1xPBS and wash for <u>3min.</u>
- 9. Meanwhile, prepare the slide chamber by underlining it with a wet paper towel.
- Remove the slides from 1xPBS and add 200µl/slide of Blocking soln. Cover with 24x 50mm cover glass, transfer to the slide chamber, and incubate at 37°C for <u>45min-1hr</u>.
- 11. Dilute the primary antibody with the washing soln. and store on ice or at 4°C.
- Remove the cover glass and add 30µl of the diluted primary antibody to each well. Cover with 22x22mm cover glass, transfer to the slide chamber, and incubate at 37°C for <u>1-1.5hr</u>.
- 13. Prewarm the washing soln. and a clean coplin jar in a 45°C water bath.
- 14. Remove the cover glass and wash <u>3X 5min</u>, in 45°C Washing soln. at RT.
- 15. Meanwhile, dilute the secondary antibody with the washing soln.
- 16. Add 30ul of the diluted secondary antibody to each well. Cover with 22x22mm cover glass, transfer to the slide chamber, and incubate at 37°C for <u>1hr</u>
- 17. Cover a coplin jar with aluminum foil to protect the slides from the light.

 $^{^1}$ Slides can be stored in 1XPBS/0.01% NaN3 after this step.

18. Remove the cover glass and wash <u>3X 5min</u> in 45°C Washing soln. at RT in the covered jar.

*If you want to stain with DAPI : add 30µl of DAPI (0.1µg/ml in 1X PBS), cover with 22x22mm and cover glass and incubate at RT for 2min. Wash 2X3min. with 1X PBS.

- 19. Shake off excess liquid from the slides.
- 20. Add 10µl of the antifading soln. to each well.
- 21. Cover with cover glass and seal with a nail polish.
- 22. Store in a slide container at 4°C.

Comments:

- 1. 1% phenylenediamine-dihydrochloride or isopropylgallate can be substituted for DABCO in the mounting medium. DABCO is harmful if swallowed, inhaled, or absorbed through the skin. Wear goggles and gloves and work in a chemical fume hood.
- 2. Alternative mounting media compositions are listed on page 11.35 of the Spector, et. al. laboratory manual.

Antibody dilutions

			IF	
Name	Maker	Source	1'	2'
Anti-20S	Affinity Research (PW 8155)	Rabbit	1:250 (CH)	1:100 (CH)
FLAG (D8)	Santa Cruz (Sc-807)	Rabbit	1:50 (SK)	
FLAG (M2)	Sigma (F-3165)	Mouse	1:250 (CH)	1:100, 1:50 TriTC (CH)
FLAG (M5)	Sigma (F-4042)	Mouse	1:500 (SK) 1:250 (CH)	1:100 (SK) 1:50 TriTC (CH)
GFP (8363-2)	Stressgen	Rabbit	N/A	N/A
GFP (8367-1)	Clontech	Rabbit	N/A	N/A
HD (HP-1)	McDonald lab	Rabbit	1:500 (SK, CH)	1:100 (SK, CH)
Hdj1 (CL#25)	RM	Mouse	1:250 (SK)	1:100 (SK)
Hdj2	Neomarker (MS-225-PO)	Mouse	1:50 (SK)	1:100 (SK)
HP1	M. MacDonald	Rabbit	1:500 (CH)	1:100 (CH)
HSF1 (1A)	RM	Rabbit	1:300 (SK)	1:100 (SK)
Hsp27	Affinity Bioreagents (MA3-015)	Mouse	1:50 (SK)	1:100 (SK)
Hsp70 (3A3)	RM	Mouse	1:500 (SK) 1:300 (GM)	1:100 (SK, GM)
HSP70 (SPA182)	Stressgen	Rabbit	1:400 (SK)	1:100 (SK)

References:

Jolly C et al. *J Histochem Cytochem* **45**, 1585-92 (1997) Kim et al. *Nat Cell Biol* **4**, 826-31 (2002)

Submitted by:

Carina Holmberg, Gen Matsumoto, Soojin Kim : May 23, 2003