



## This kit includes:

25 ml Silver-solution  $\text{AgNO}_3$  (5%), 10 ml Citric acid (0.5%)

## Additionally needed:

Trichloroacetic acid (TCA), NaOH (1M), ammonia (25%), glutardialdehyde (25%), formaldehyde (37%), methanol (MeOH), acetic acid (HAc), Sodium acetate (NaAc), ethanol, glycerol (85%), distilled water. All chemicals are of p.A.quality

## Solutions and Ports

### (Port 1): Fixing solution (20%TCA)

40 g TCA, dissolve in distilled water and make up to 200 ml.

### (Port 2): Rinsing I (50% MeOH, 10% HAc)

Add 100 ml MeOH and 20 ml HAc and make up to 200 ml with distilled water.

### (Port 3): Rinsing II (5%MeOH, 7% HAc)

Add 10 ml MeOH and 14 ml HAc and make up to 200 ml with distilled water.

### (Port 4): Incubation: (2.5% Glutardialdehyde, 1.75% NaAc)

Add 20 ml Glutardialdehyde-solution (25%) and 3.5g NaAc to 180 ml with distilled water.

### (Port 5): Hot Water

800 ml, 1.4 l if only cold water is used (then cancel port 9).

### (Port 6): Ammoniacal silver reagent

*Prepare this solution freshly before use and inside of an exhaustor!* To 50 ml distilled water add 4 ml 1M NaOH, then add 1.7 - 2.0 ml ammonia-solution (25%) and slowly add 5 ml of the **silver-solution (5%)**. (Any brownish precipitate should immediately disappear, if not the ammonia-solution is too old!). Make up to 200 ml with distilled water

### (Port 7): Developing solution

*Prepare freshly before use:* Take 1 ml of the **citric acid-solution (0.5%)** and make up to 200 ml with distilled water. Add 100  $\mu\text{l}$  of the formaldehyde (37%)

### (Port 8): Stopping and Preserving Solution (10% MeOH, 1% HAc, 5% Glycerol)

Add 60 ml MeOH and 30 ml Glycerol and 6 ml HAc and make up to 600 ml with distilled water.

## Staining Programme

step	reagent	volume	temp	time
1. Fixing	20% trichloroacetic acid (w/v)	200 ml	20 °C	30 min
2. Washing	H <sub>2</sub> O dist	200 ml	20 °C	5 min
3. Rinsing I	50 % methanol / 10% acetic acid (v/v)	200 ml	20 °C	30 min
4. Rinsing II	5% methanol / 7% acetic acid	200 ml	20 °C	15 min
5. Incubation	2.5 % glutardialdehyde	200 ml	20 °C	15 min
6. - 9. Washing	H <sub>2</sub> O dist (optional: Hold overnight in the last step)	3 x 200 ml	20 °C	20 + 15 + 10 + 10 min
10. Silvering (Prepare freshly before use!)	Silver Solution	200 ml	20 °C	30 min
11.-12. Washing	H <sub>2</sub> O dist (cold!)	2 x 200 ml	20 °C	1 + 5 min
13. Developing (Prepare freshly before use!)	Developing Solution	200 ml	20 °C	10 - 15 min observe to stop (Set Beep!)
14.-16. Stopping & Preserving	10% Ethanol, 1% Acetic Acid, 5% Glycerol	3 x 200 ml	20 °C	10 + 10 + 10 min



Silver-staining according to Dr. Wurster's manual:

### **"Detektion von oligoclonalem IgG in Liquor und Serum"**

GE: Application Note #1.

If this Silver-Staining Kit is used the recipes below are valid, not of Dr. Wurster's manual!

If non-heated solutions are used, the times in Dr. Wurster's manual should be used.