

C37 } use as 1:20 dil. in EDTA buffer  
C34 }  
C38 }

## **METHODOLOGY FOR ANTIGEN RETRIEVAL**

### **HEAVY MAR : ( use of pressure cooker)**

- In the microwave pressure cooker, add the appropriate solution (verify on the antibody list on top of the shelf) with the appropriate pH
- Put the shelf into the cooker. Make sure perforated shelf is curve down/handles up
- Place slides into upright (Tissue-Tek) slide racks and place onto the cooker shelf
- Close cooker , making sure that "O" ring is properly seated before locking the lid
- Cook on HIGH until the yellow valve pops-up, then cook for an additionnal 2 minutes (full pressure)

**For EDTA buffer :            in our microwave ( 32 minutes)**

**For CITRATE buffer:        in our microwave ( 27 minutes)**

**\*\*\*ALWAYS USE A FULL RACK AND CENTER IT IN THE COOKER. (Use blank slides if necessary) IF YOU USE TWO, CENTER THEM AS WELL**

### **LIGHT MAR : ( no pressure cooker)**

CY68 - This Buffer  
at 1:50

- Prepare buffer as recommended. Hydrate the slides to be retrieve and place in an upright slide rack (Tissue-Tek).
- Place a plastic Technicon staining dish in the styrofoam box filled cooler
- Boil the buffer ( usually 4 minutes at HIGH power) in the microwave.
- Then pour the boiling buffer into dish.
- Transfer slides, cover cooler
- Let stand for 30 minutes then remove the dish from the cooler and place into cold running tap water
- Place slides in APK was buffer and continue staining

# **BUFFER'S RECIPES FOR ANTIGEN RETRIEVAL**

## **TRIS BUFFER (0.75 M)** (light MAR)

- **STOCK SOLUTION:**

90.86 g of Tris base in 1 liter of distilled water.  
Adjust pH at 10.0 with 2N HCL/ or 0.1 N NaOH

- **WORKING SOLUTION:**

20 ml of stock solution + 280ml of distilled water  
Adjust pH at 10.0 as above

## **CITRATE BUFFER (10mM)** (pressure cooker)

- **STOCK SOLUTION:**

31.5g of citric acid in 1 liter of distilled water  
Adjust pH at 6.0 with 2N HCL/ or 0.2N HCL

- **WORKING SOLUTION:**

100ml of stock solution + 1400ml of distilled water  
Adjust pH at 6.0 with 2N HCL/ or 0.2N HCL

## **EDTA BUFFER (0.1M)** (pressure cooker)

- **STOCK SOLUTION:**

37.2 g of EDTA powder in 1 liter of distilled water  
Adjust pH at 8.0 (as above)

- **WORKING SOLUTION:**

150ml of stock solution + 1350ml of distilled water  
Adjust pH at 8.0 (as above)

P.S: EDTA= ethylenediamine-tetraacetic acid

# MICROWAVE ANTIGEN RETRIEVAL (MAR)

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## SOLUTIONS

**15x TRIS BUFFER:** 90.86g Tris base in 1 litre dH<sub>2</sub>O, (0.75M). Store in a closed container in fridge.

**Working Tris:** a) 100ml 15x concentrate + 1400 ml dH<sub>2</sub>O. Adjust pH to 10.0 with approximately 1.5-2.0 ml. of 2N HCl

b) 20ml 15x concentrate + 280ml dH<sub>2</sub>O; adjust to pH10.

**OR**

**15x CITRATE BUFFER:** 31.5g Citric Acid monohydrate (H<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>H<sub>2</sub>O) 1L distilled water. Adjust pH to 6.0 with 10N NaOH (or Na Citrate)

**Working Citrate:** 100ml 15x Citrate + 1400 ml dH<sub>2</sub>O

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## 1) "HEAVY" MAR

In the microwave pressure cooker, add 100ml. 15X buffer and 1400ml dH<sub>2</sub>O, adjust the pH; put the shelf into the cooker. Place slides into upright (Tissue-Tek) slide racks and place onto the cooker shelf.

**NB: IT IS IMPORTANT THAT THERE ALWAYS BE THE SAME NUMBER OF SLIDES AND SAME AMOUNT OF SOLUTION (ie the same geometry) IN THE COOKER.... THEREFORE ALWAYS USE TWO FULL RACKS (48 SLIDES - INCLUDE BLANKS TO MAKE-UP 48 SLIDES) IN 1500ML OF 1X BUFFER.**

**Make sure perforated shelf is curve down/handles up.**

Close cooker making sure that "O"-ring is properly seated before locking the lid; cook on HIGH until the yellow valve pops-up, then cook for 30 seconds to 1 min. longer (**IN OUR OVEN - 18 MIN. TOTAL**). Remove from microwave and **let stand for 10 min.** Cool cooker in cold running water until buffer is tepid (**LEAVE at least 1 HOUR - 90 min. best**). Water rinse slides then place in appropriate buffer X3 for 10 min. total and continue staining.

## 2) "LIGHT" MAR

Prepare a 1X buffer as above and adjust the pH. Hydrate the slides to be "retrieved" and place in an upright slide rack (Tissue-Tek). Place a plastic Technicon staining dish in the styrofoam filled cooler, (when ready to proceed put slide rack into staining dish). Boil the 1X buffer and pour the boiling buffer over the slides. Cover the dish; cover the cooler; let stand 30 min.. Remove the dish from the cooler and place into cold running water until buffer is tepid, (20 min). Water rinse the slides (less than 1 min); buffer wash the slides for 10 min; continue with staining.

# MICROWAVE ANTIGEN RETRIEVAL (MAR)

K.Kemball 950525

## SOLUTIONS

**15x TRIS BUFFER:** 363.42g Tris base 4 litres dH<sub>2</sub>O, (0.75M).  
Store in a closed container in fridge.

**Working Tris:** a) 100ml 15x concentrate + 1400 ml dH<sub>2</sub>O.  
Adjust pH to 10.0 with approximately 1.5 - 2.0 ml of 2N (normal) HCl  
b) 20ml 15x concentrate + 280ml dH<sub>2</sub>O;  
adjust to pH10

**OR**

**15x CITRATE BUFFER:** -31.5g Citric Acid monohydrate (H<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>H<sub>2</sub>O) L distilled water  
-Adjust pH to 6.0 with 10N NaOH (or Na Citrate)

**Working Citrate:** 100ml 15x Citrate + 1400 ml dH<sub>2</sub>O

### 1) "HEAVY" MAR

In the microwave pressure cooker, add 100ml 15x buffer and 1400 ml dH<sub>2</sub>O, adjust the pH; put the shelf into the cooker. Place slides into upright (Tissue-Tek) slide racks and place onto the cooker shelf.

**NB: IT IS IMPORTANT THAT THERE ALWAYS BE THE SAME NUMBER OF SLIDES AND SAME AMOUNT OF SOLUTION (i.e. the same geometry) IN THE COOKER... THEREFORE ALWAYS USE TWO FULL RACKS (48 SLIDES - INCLUDE BLANKS TO MAKE UP 48 SLIDES) IN 1500 ML OF 1X BUFFER.**

Cook on HIGH until the yellow valve pops up, then cook for 30 sec. longer (IN OUR OVEN - 18 MIN. TOTAL). Remove from microwave and let stand for 10 min. Cool cooker in cold running water until buffer is tepid (LEAVE AT LEAST 1 HOUR). Water rinse slides then place in appropriate buffer X3 for 10 min total and continue staining.

### 2) "LIGHT" MAR

Prepare a 1X buffer as above and adjust the pH. Hydrate the slides to be "retrieved" and place in an upright slide rack (Tissue-Tek). Place a plastic Technicon staining dish in the Styrofoam filled cooler, (when ready to proceed, put slide rack into staining dish). Boil the 1X buffer and pour the boiling buffer over the slides. Cover the dish; cover the cooler; let stand 45 min.. Remove the dish from the cooler and place into cold running water until buffer is tepid. Water rinse the slides; buffer wash the slides for 10 min; continue with staining.

## SOLUTIONS:

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### TRIS BUFFER 0.5 M STOCK is 10 X (Light MAR)

- 60.6 Gram Trizma Base /Liter ddH<sub>2</sub>O adjust to ph 10.0 and autoclave.

### Working solution (0.05 M):

- Dilute 1:10 in ddH<sub>2</sub>O and adjust to ph 10.0

### Citrate Buffer (100mM) Stock is 10 X: (Pressure Cooker)

- Gram Citric Acid anhydrous/Liter ddH<sub>2</sub>O adjust to ph 6.0 with 10 N NAOH and autoclave

### Working solution (10 mM)

- Dilute 1:10 in ddH<sub>2</sub>O

### EDTA Buffer (0.1 M ) Stock is 10 X (Pressure Cooker)

- 37.2 Gram of EDTA (ethylenediamine-tetraacetic acid) per 1 Liter dd H<sub>2</sub>O Adjust ph to 8.0 and autoclave.

### Working solution ( 0.01 M or 100 mM)

- Dilute 1:10 in dd H<sub>2</sub>O and adjust to ph 8.0