

## List of Buffers

### Carbonate Buffer for coating ELISA

0.1 M Na<sub>2</sub>CO<sub>3</sub> adjust pH to 9.6 with 0.1 M NaH<sub>2</sub>CO<sub>3</sub>

### Coagulation buffer

A drop of EDTA 100mM in an eppendorf tube that will receive the blood is enough.

### Complete culture RPMI Medium

Gibco RPMI 1640 with L-Glutamine (Ref 21875) -->500ml

All from Gibco:

- + Glutamine 2mM (if the medium do not contain any) --> 5ml
- + Sodium Pyruvate 100mM (Ref 11360) (100x) --> 5ml
- + MEM Non Essential Aminos Acids (Ref 11140) (100x) --> 5ml
- + Hepes 1M (Ref 15630) (100x) --> 5ml
- + Peni-Strep (Ref 15140) (100x) --> 5ml
- + Gentamycin 50mg/ml (Ref 15750) (1000x) --> 500ul
- + βMercaptoethanol 50mM (Ref 31350) (1000x) --> 500ul
- + FCS 10% --> 50ml

### Confocal Buffer

PBS, 10%, FCS, 0.1% Azide

### DABCO (immunomount pour slide pour confocal)

90% glycerol

10% PBS

2.5g/100ml DABCO [1,4-Diazabicyclo(2,2,2)octane]

Dissolve de DABCO first in PBS (azide at 0.1/0.05%), then add the glycerol little by little while shaking.

Adjust the pH to 8.6 by adding 2NHCl ~ 1 to 2ml (or 800 µl of 12N HCl in 100ml).

### FACS Buffer

PBS, 0.1% (w/v) sodium azide, 1% FCS, EDTA 2mM

### Immunohistochemistry Buffer - Tris pH7.6

1 part of TRIS 0.2M (121.14g / 5L)

1.5 part of NaCl (42.5g / 5L)

1.5 part of 0.1N HCl (50ml / 5L)

### T cell *in vitro* stimulation

PMA (2. Phorbol 12-Myristate 13-Acetate) 50ng/ml and Ionomycin 1µg/ml

4h for cytokines intracellular staining; 24h for cytokines ELISA