

## **FIXATION PROTOCOL platelets**

### **Whole blood fixation procedure**

Fixative:                                2.4% glutaraldehyde  
    0.24% freshly prepared paraformaldehyde  
    0.1 M phosphate buffer

Collect whole blood directly from the vein into fixative (1:5 v/v - blood/fixative)  
Fix cells for 30 min at room temperature and then remove the fixative by centrifugation  
Replace by normal concentrated (2%GA +0.2 PFA) and fix for another 2hr at RT or overnight at 4°C  
Replace by storage fixative (1% PFA in 0.1 M phosphate buffer)  
The cells are ready to be sent. Eppendorf should be completely filled with the storage fixative and extra sealed with parafilm.

### **Platelets in citrated PRP**

Fixative (two times concentrated):    0.4% glutaraldehyde  
    4% freshly prepared paraformaldehyde  
    0.1 M phosphate buffer

Do not wash the platelets but gently add a volume of fixative that is equal to the volume of the PRP (end-concentration will then be 0.2% glutaraldehyde + 2% formaldehyde).  
Fix cells for 30 min at room temperature and then remove the fixative and replace it with 0.2% glutaraldehyde, 2% paraformaldehyde, 0.1 M phosphate buffer  
Fix for another 2 h at room temperature or overnight at 4°C  
Rinse cells once with 0.1 M phosphate buffer  
Replace by 1 % paraformaldehyde in 0.1M phosphate buffer. Eppendorf should be completely filled with the storage fixative and extra sealed with parafilm.