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.