PROTOCOL FOR SAMPLE PREPARATION FOR PROTEOMIC ANALYSIS

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Platelet concentrate sample preparation:

1. We normally use 'bullet' Eppendorf-style tubes for these preparations.
2. Collect 4 x 1 mL aliquots of the platelet concentrate and spin at 200 x g for 10 minutes at room temperature. Remove the supernatant platelet-poor plasma for further preparation as described in the next section below (platelet releasate preparation)
3. Wash the platelet pellet by resuspending with room temperature CGS buffer (10 mM sodium citrate, 30 mM dextrose, 120 mM sodium chloride, adjusted to pH6.5), then spin at 200 x g for 10 minutes at room temperature. Remove supernatant and discard.
4. Freeze pellet at -80°C.
5. Please provide platelet count of starting platelet concentrate, if available

Platelet releasate sample preparation:

1. Begin from the 4 supernatant plasma samples from the platelet concentrate sample preparation described above. Keep samples in individual tubes.
2. Spin tubes at 1000 x g for 20 minutes at room temperature.
3. Transfer non-turbid supernatants into four new bullet tubes and freeze at -80°C.

Red cell concentrate sample preparation:

1. Using a Eppendorf tube (also known as a bullet tube), collect 2 samples of 200 µL of red cell concentrate and spin at 500 x g for 10 minutes at 4°C. Remove supernatant and discard.
2. Wash red cell pellet by resuspending in 1 mL cold saline, then spin at 500 x g for 10 minutes at 4°C.
3. Remove supernatant fluid and discard. Freeze pellet at -80 °C.
4. Please provide concentration of RBC in the original red cell concentrate unit, if known.