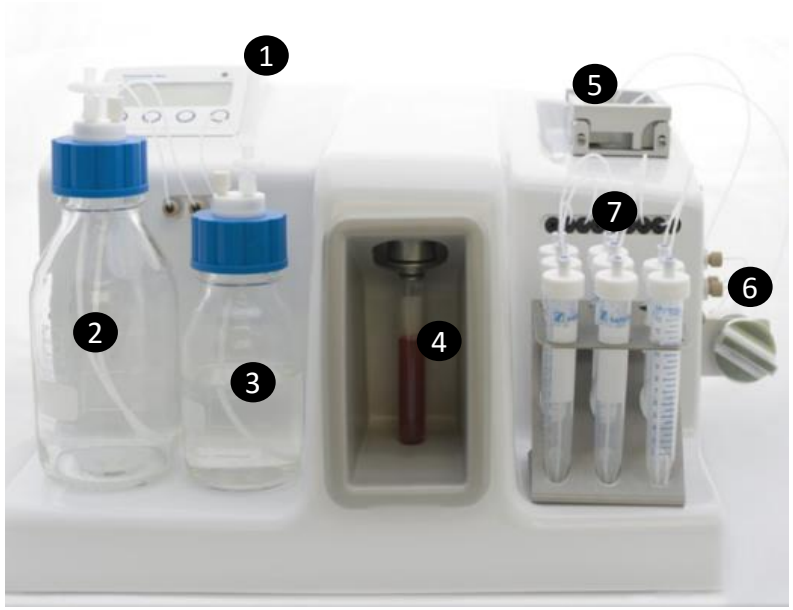
ANGLE

Parsortix™ PR1

For Research Use Only

Quick Reference Guide



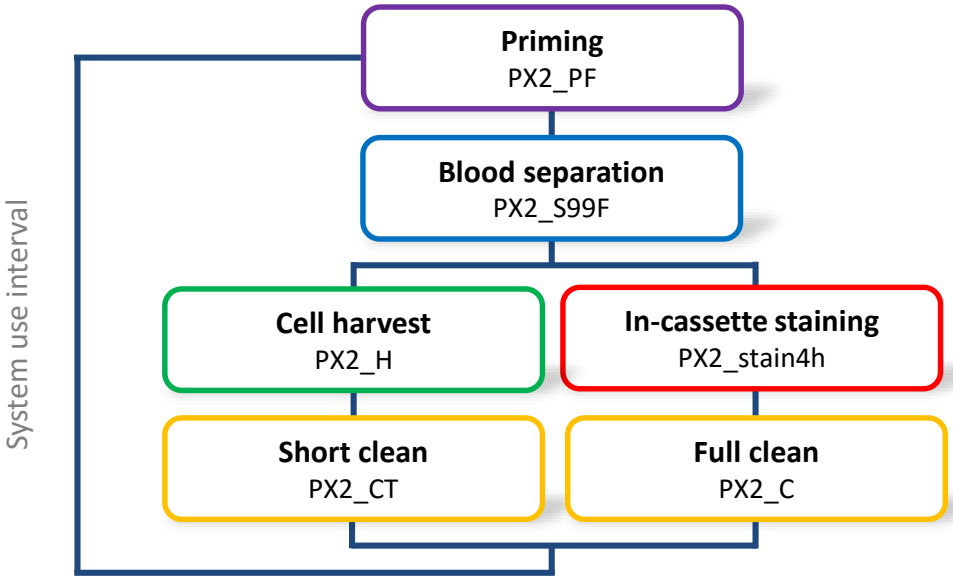
System components

1. Display and control panel
2. Waste reservoir
3. Buffer reservoir
4. Sample vacutainer
5. Cassette clamp
6. Harvesting valve
7. Reagent lines and tubes:

Before you start, check….

- ☑ Blood drawn into **EDTA vacutainer**, less than **48 hours old**, stored at 4°C.
- ☑ Blood allowed to equilibrate to room temp on a roller mixer for 20mins.
- ☑ Harvest waste tube is empty and the line sits **less than 8cm** inside the tube.
- ☑ Ensure lines in cleaning and priming tubes reach the bottom of the tubes.
- ☑ The **harvesting valve** is turned **clockwise** in the 'SEP' position.
- ☑ **Reagents to be filled before cleaning protocol; volumes to be no lower than….**
  - 100 ml PBS in buffer reservoir
  - 20 ml LabKlenz 110 (10%) in cleaning solution tube (label C)
  - 20 ml ethanol (100%) in priming solution tube (label P)
  - 50 ml Bleach in waste reservoir (note: empty waste when it reaches 400 ml and replace Bleach)

Parsortix™ Workflow



Priming

1. Select protocol *PX2_PF* and press [Run] then [Start].
2. On prompt insert a new cassette.
2. Press [OK] to start the priming process.
3. When the process is finished (after ~15 min), press [OK] then [Continue].

Blood separation

1. Select protocol *PX2_S99F* and press [Run] then [Start].
2. At prompt "Rinse vacutainer", partially remove the 50ml tube and press [OK] to start the rinse, collect the fluid in the tube.
3. Remove the tube, avoid flicking fluid off the tip of the line.
4. At prompt "Attach Vacutainer", invert the sample several times to resuspend the blood cells, immediately mount onto the machine and press [OK].
5. At prompt "Start?", press [OK] to start the separation.
6. After approx. 30min, resuspend the settled blood cells by tapping the Vacutainer while it sits in the inclined position.
7. When the process is finished (after ~2.0 h for a 10 ml sample), press [OK] then [Continue].

Cell harvest

1. Select protocol *PX2_CT2* and press [Run] then [Start].
2. At prompt "Insert cleaning cassette", remove the separation cassette and insert the cleaning cassette, press [OK].
3. At prompt "Empty rgt tubes", ensure reagent tubes are empty and press [OK].
4. When finished (after ~20min) press [OK] then [Continue].
5. **Remove cleaning cassette and reinsert separation cassette.**
6. Select protocol *PX2_H* and press [Run] then [Start].
7. When prompted, rotate the harvest valve anticlockwise to the position 'HAR' and press [OK].
8. At prompt "Start", remove the harvest line from the harvest waste tube and clean it with an alcohol wipe, wait until dry.
9. Place a collection vessel (e.g. an Eppendorf tube) beneath the harvest line.
10. Press [OK] to start the harvest. 200µl will flow through the line. If required, press YES to collect a further 1 ml or No if not.
5. On prompt, rotate the harvest valve clockwise to the position 'SEP' and press [OK]. Return harvest line to the tube.
6. Press [OK] then [Continue].

In-cassette staining

Note: Please reference 'Immunofluorescence staining for in-cassette and on Parsortix™ harvest' guide for detailed instruction. Ensure system has been fully cleaned using PX2_C prior to blood separation

1. Add the following reagents to fresh clean 15 ml Falcon tubes:
    - Line 1: 2 ml 4% formaldehyde
    - Line 2: 2 ml 0.1% Triton X-100 in PBS
    - Line 3: 1 ml blocking buffer
    - Line 4: 1 ml primary antibodies (at optimised concentration in blocking buffer)
    - Line 5: 1 ml secondary antibodies (at optimised concentration in blocking buffer containing 1:5000 DAPI)
2. Select protocol *PX2_stain4h* and press [Run] then [Start].
3. When finished press [OK] then [Continue].

Cleaning

1. Check reagent levels. Minimum reagent volumes can be found on the reverse of the sheet in "Before you start" section.
2. Select the appropriate cleaning protocol according to the flowchart and press [Run] then [Start].
3. At prompt "Insert cleaning cassette", remove the separation cassette, insert a cleaning cassette and press [OK].
4. At prompt "Empty rgt tubes", empty the reagent tubes if necessary and press [OK] to start the cleaning cycle.
4. When finished press [OK] then [Continue].
5. Empty the fluids from the harvest waste tube.
6. In the case of a full clean process (PX2_C), discard the reagent tubes 1-6, and clean the outside of the lines with an alcohol soaked wipe to remove residual cleaning fluid. Replace the reagent tubes with new ones.
7. Remove the vacutainer from its mount and discard safely.
8. Clean the O-ring and the outside of the line using an alcohol soaked wipe.
9. Place a 50 ml Falcon tube on the mount. This tube collects rinse fluid in the separation process and can be reused.

Customer support

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