Application Note

Cytospin Slide Preparation from Parsortix® Harvest

Introduction

THE PROCEDURES DESCRIBED IN THIS DOCUMENT ARE FOR RESEARCH USE ONLY AND NOT FOR USE IN CLINICAL OR DIAGNOSTIC PROCEDURES.

The Cytospin is a centrifuge that concentrates and deposits a thin-layer of cells within a clearly-defined area on a microscope slide while maintaining cell integrity. The method described within was developed to lay cells from the Parsortix harvest onto a slide using the Cytospin 4 centrifuge along with the Shandon Cytofunnel™ and Cytoslides™. The prepared slides from the Parsortix harvest can be used in numerous cytological and immune staining assays (e.g. Wright Giemsa; fluorescent in situ hybridization, FISH; immunocytochemistry, ICC).

IMPORTANT: The preparation of Cytoslides from the Parsortix harvest can lead to cell loss; the cell loss needs to be assessed by users on a case-by-case basis.

Equipment, Reagents and Consumables

Equipment and consumables

Equipment	Catalogue Number	Manufacturer
Cytospin® 4 Cytocentrifuge	A78300003	ThermoFisher
Parsortix® PR1 Instrument	-	ANGLE
Parsortix® Cell Separation Cassette	GEN3P6.5	ANGLE
Parsortix® Cleaning Cassette	GEN3P10/GEN3D10	ANGLE
BD K_2EDTA Vacutainer® blood collection tube	Cat no. 366643 (US) or 367525 (EU)	BD (Beckton Dickinson)

Reagents

Reagents	Catalogue Number	Manufacturer
Shandon™ EZ Single Cytofunnel™ with White Filter Cards and Caps	A78710003 or A78710020	Fisher Scientific
Shandon™ Single Cytoslides™, coated, circle on back	12070266	Fisher Scientific
Fetal Bovine Serum (heat inactivated, filtered)	10437010	Fisher Scientific or equivalent
100% Methanol	34860	Sigma-Aldrich or equivalent
LoRetention Dualfilter Tip (50 - 1000 µl)	EPPE0030077.652	VWR or equivalent
Eppendorf™ LoBind 1.5mL Microcentrifuge Tubes - Protein	13-698-794	Fisher Scientific or equivalent

Methods

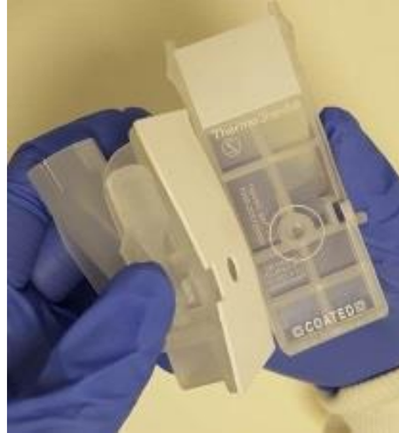
Parsortix Harvest and Cytospin Slide Preparation

IMPORTANT: Gloves must be worn when handling the positively charged glass slides and/or Cytofunnel Assembly to prevent the transfer of skin cells onto the slides.

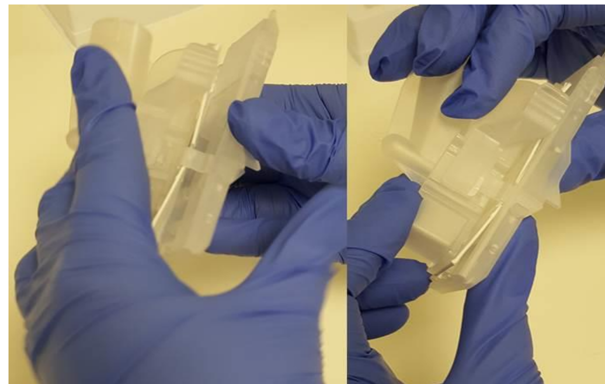
- Upon completion of blood separation with the Parsortix PR1, proceed to harvest (**PX2_H**). Carefully wipe the external instrument tubing of the harvesting line with an alcohol soaked wipe. Ensure the harvest line is completely dried before proceeding with the harvesting.
- Harvest cells captured in the cassette directly into a 1.5mL LoBind Eppendorf tube containing 60µL of filtered Heat Inactivated Fetal Bovine Serum (HI-FBS). Avoid touching the HI-FBS and the side of the microfuge tube with the harvest output line. Ensure the final drop of liquid from the harvest is deposited into the microfuge tube by gently flicking the harvest line while it is still inside the microfuge tube.
- Proceed without delay to the cytocentrifugation process outlined below.
- Label slide as required with a solvent proof marker (e.g.: pencil or Cryoware pen).
- While wearing gloves, remove a Cytofunnel from its container without touching the white filter paper as shown below:

- Insert the properly labeled Cytoslide with the coated side up into the groove of the Cytofunnel assembly as shown below, being careful not to touch the white paper filter or coated portion of the slide.

- Carefully fold the top portion of the Cytofunnel assembly onto the slide, taking care not to touch the glass slide or paper filter. DO NOT apply any force on the release latch.



- Once the Cytofunnel assembly is completely folded over, position your finger in the vicinity of the click and lock system and gently push shut. A subtle clicking noise will confirm that the Cytofunnel assembly has been properly locked. While holding the bottom of the Cytofunnel assembly, gently pull upwards on the funnel to ensure that the mechanism has locked securely.



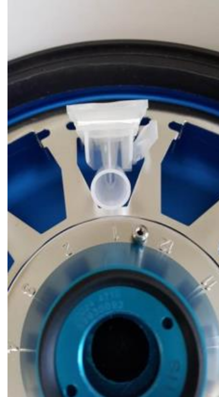
IMPORTANT: Use caution when handling the Cytofunnel Assembly to ensure that you DO NOT depress the Release Lever. Pressing the Release Level will open the Cytofunnel Assembly, rendering it useless.

- Proceed with inserting the Cytofunnel assembly into the Cytospin 4 cytocentrifuge.

  NOTE: If running only one sample, insert a second Cytofunnel assembly containing a slide as a balance. If running multiple samples, similarly ensure these are balanced.

- Set a 1000µL pipette to 290µL and attach a clean LoRetention Dualfilter tip.

- **Slowly** rotate the Cytospin rotor until the appropriate Cytofunnel™ assembly containing the properly labeled Cytoslide is facing you as shown below:

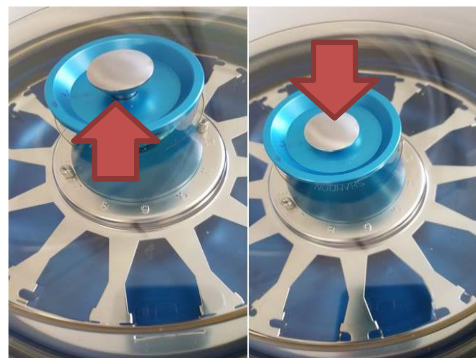


- Draw up the entire contents of the microfuge tube into the pipette LoRetention Dualfilter tip and carefully place the pipette LoRetention Dualfilter tip into the bottom of the Cytofunnel, as illustrated below:

- Slowly and steadily depress the plunger of the pipettor to transfer the entire harvest/FBS mixture into the Cytofunnel assembly while it remains in the Cytospin rotor.

IMPORTANT: Be sure to keep the pipette tip in the bottom of the Cytofunnel on the side away from the slide as shown above. Do not tilt the Cytofunnel assembly; keep it in its position inside the rotor to avoid the liquid touching the slide prior to centrifugation. Make sure the liquid does not touch the conical sides of the Cytofunnel. Take care to minimise the number of bubbles within the harvest FBS mixture as this may affect cell transfer.

- Pull up on the central knob of the rotor cover lid and position it on top of the rotor. Secure the lid onto the rotor by depressing the central knob (marked by the arrow).

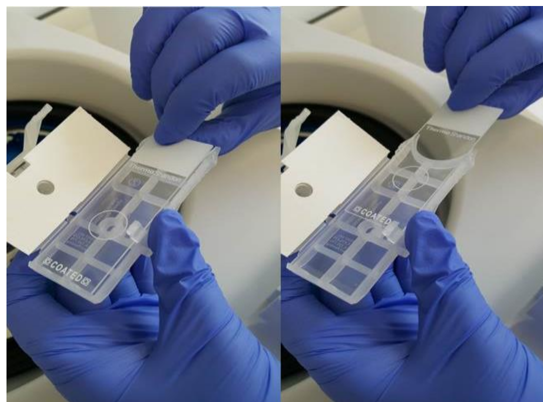


- Set the time and centrifuge speed to 3 minutes and 800 RPM, and ensure the "Low Acceleration" option is selected.

- Press the "Start" button to start the Cytospin.
- Audible warning tones will sound and error codes will be displayed if the Cytospin detects a situation that prevents it from working safely and efficiently. In this situation, refer to the Cytospin Operator Guide (Table 2) when error codes are observed and attempt remedies suggested where possible.
- Once the Cytospin has stopped, open the lid of the cytocentrifuge and remove the cover from the rotor by pulling up on the central knob of the rotor cover lid.
- Carefully remove the Cytofunnel assembly from the rotor, and open it by pressing on the release lever and lifting up the top portion of the Cytofunnel assembly as shown below.

IMPORTANT: Gloves must be worn when handling the positively charged glass slides to prevent the transfer of skin cells onto the slides.

- Carefully remove the Cytoslide from the Cytofunnel assembly as shown below:



- Allow the slide to air dry for one (1) minute on a clean paper towel.
- Immediately processed with slide fixation. Immerse the slide, label towards the top, into a Coplin jar containing a sufficient volume 100% methanol fixing agent such that the liquid level covers the slide and is at or near the frosted label portion of the slide.
- After one (1) minute remove the slide from the fixing agent, gently tap edge of the slide on a paper towel to remove excess liquid, and then place the slide on a clean paper towel on flat surface and allow to the slide to air dry at room temperature for 30 minutes.

  Note: Other type of fixative used, and the fixation time will require optimization. Alternative fixative conditions need to be assessed case-by-case by users.

- Cytoslides are stable up to one (1) week when stored at room temperature protected by direct light and dust.

  Note: Cytoslide stability needs to be assessed case-by-case by users.

Sample Outcomes

Example of a cytological slide (e.g. Wright-Giemsa)

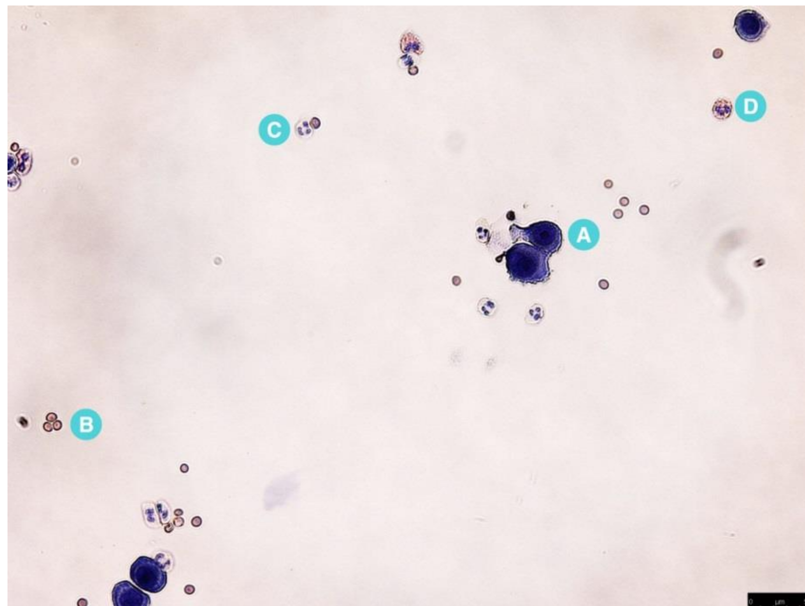


Figure 1: Representative image of a Parsortix harvest cytoslide stained with a three-step stain kit/Wright-Giemsa. Blood was collected in a K_2EDTA tube (BD cat no. 366643 (US) or 367525 (EU)), spiked with SKBR3 cells, and processed within 4 hours with the Parsortix® PR1. The harvest was transferred onto a cytoslide as described in the above method. The cytoslide was stained with the "Three-step stain kit" (500mLx3, ThermoFisher Scientific, cat no. 3300) equivalent to the Wright-Giemsa staining. Scale bar 50 µm; an example of **A)** SKBR3 cell; **B)** erythrocyte; **C)** neutrophil, and **D)** eosinophil can be viewed in the representative image.

Example of a FISH slide (HER2/neu and CEP17 FISH)

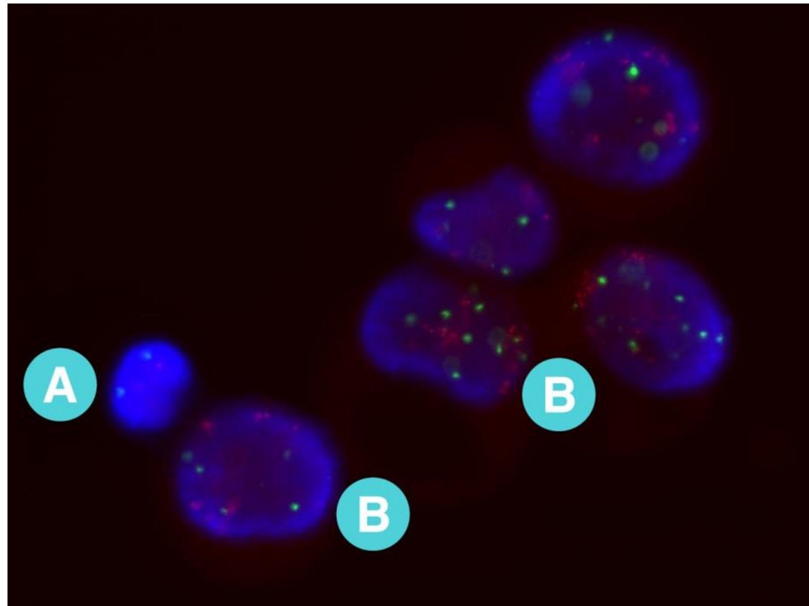


Figure 2: Representative image of a HER2/neu and CEP17 FISH performed on a Parsortix harvest cytoslide. Blood was collected in a K_2EDTA tube (BD cat no. 366643 (US) or 367525 (EU)), spiked with SKBR3 cells, and processed within 4 hours with the Parsortix® PR1. The harvest was transferred onto a cytoslide as described in the above method and with a 15 min methanol fixation. The FISH was performed according to the hybridization procedure as described in https://www.molecular.abbott/sal/30-608377.pdf. LSI HER-2/neu SpectrumOrange / CEP17 SpectrumGreen part number: 30-171060 / 35-171060, (PathVision, Molecular Abbott), was used for the FISH evaluation. An example of **A)** Nucleated blood cell and **B)** SKBR3 cell can be viewed in the representative image. Orange/red dots correspond to HER-2/neu and green dots correspond to CEP 17 hybridized probes respectively. Nuclei are stained in blue/DAPI. HER2 amplification is visible in SKBR3 cells.

Example of a fluorescence ICC staining (PanCK-CD45)

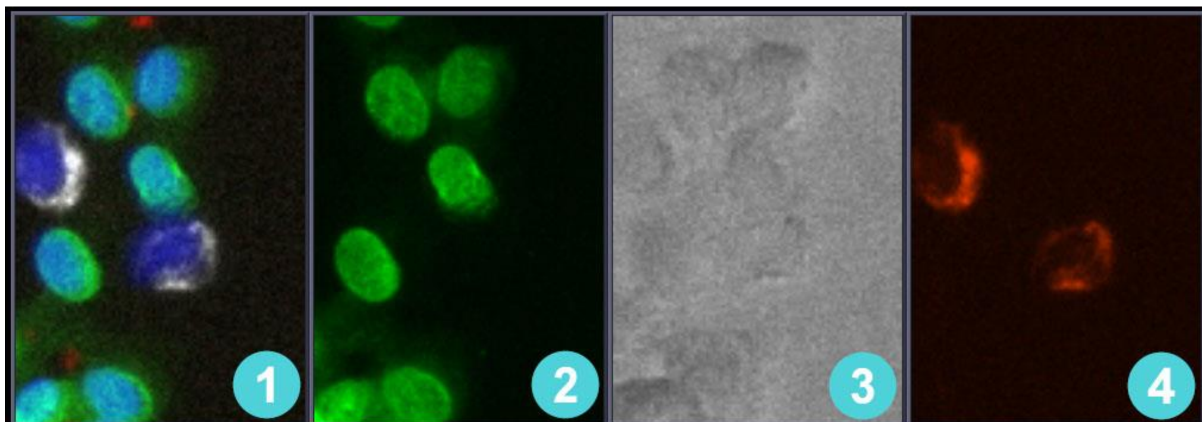


Figure 3: Representative image of a fluorescence ICC (PanCK-CD45) staining performed on a Parsortix harvest cytoslide. Blood was collected in a K_2EDTA tube (BD cat no. 366643 (US) or 367525 (EU)), spiked with SKBR3 cells, and processed within 4 hours with the Parsortix® PR1. The harvest was transferred onto a cytoslide as described in the above method, but

it was fixed with 100% ice-cold for five (5) minutes at -20°C. The cytoslide was stained with PanCK (green), CD45 (red/grey), and DAPI (blue), in panel **1)** PanCK$^+$ (green)/CD45$^-$ (grey) circulating tumour cells (CTCs), PanCK$^-$ (green)/CD45$^+$ (grey) nucleated blood cells, and DAPI$^+$ (blue) nuclei; in panel **2)** PanCK$^+$ (green) CTCs; in panel **3)** bright-field image; in panel **4)** CD45$^+$(red) nucleated blood cells.

Example of a double HRP (Brown)/AP (Red) ICC staining

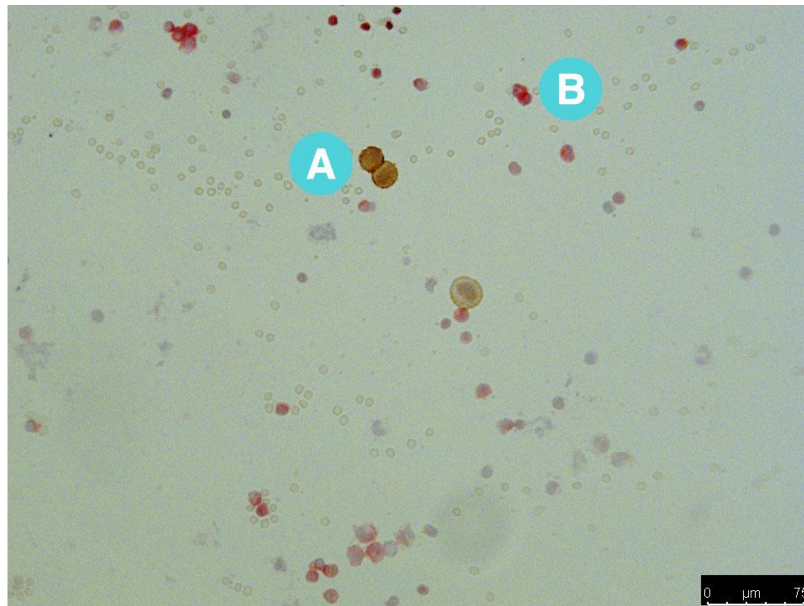


Figure 4: Representative image of an ICC (PanCK-CD45) staining performed on a Parsortix harvest cytoslide. Blood was collected in a K_2EDTA tube (BD cat no. 366643 (US) or 367525 (EU)), spiked with SKBR3 cells, and processed within 4 hours with the Parsortix® PR1. The harvest was transferred onto a cytoslide as described in the above method. The cytoslide was stained with PanCK (A1/A3, brown) and a CD45 (red) utilizing a Dako automated staining and nuclei were counterstained with hematoxylin. Scale bar 75µm; an example of: **A**) SKBR3 cell (brown) & **B)** nucleated blood cells (red) can be viewed in the representative image.

Troubleshooting

See Parsortix User Manual for separation, harvesting or general instrument troubleshooting. Contact ANGLE technical support at us-support@angleplc.com or eu-support@angleplc.com for additional support.

See Cytospin® 4 Cytocentrifuge, A78300003 (ThermoFisher) User Manual for Cytospin® troubleshooting.