ANGLE

CellKeep™ Slide Instructions for Use

Product name: CellKeep™
Product code: CLK-SET-01.B

For Research Use Only (RUO). Not for use in diagnostic procedures.

1. Product Overview

CellKeep slides are a staining support item designed to maximise the retention of rare cells such as Circulating Tumour Cells (CTCs) harvested from blood samples, processed by the Parsortix® instrument.

Rare cells, such as CTCs, are infrequent occurrences in cancer patient blood samples. As a result, the staining support item must have consistently high cell retention performance during processing to maximise the likelihood of rare cell capture for subsequent image analysis.

The CellKeep slide consists of a coated glass slide clipped into a plastic funnel assembly. A silicon gasket surrounds the sample area underneath the funnel. Cells harvested from the Parsortix instrument are deposited directly into the funnel of the CellKeep slide and centrifuged. A wicking cap is used to remove the liquid before the addition of a drying buffer. The slide is centrifuged again before a second wicking cap is used to remove the excess drying buffer. The slide is allowed to dry before fixation and storage or IF staining (not part of this instruction).

The use of CellKeep slides for the capture of rare cells has been validated via the processing of enriched samples from the Parsortix® instrument for subsequent image analysis of circulating tumour cells, which are a classification of rare cells.

2. Warnings and Precautions

Please read the entire content of these Instructions for Use (IFU) before testing samples.

Materials Safety Data Sheet (MSDS) is available on request.

The CellKeep slide should only be used with:

Cells or enriched samples harvested by a Parsortix® instrument from blood samples collected in appropriate sample collection tubes.

Warning: The population of cells and any other materials in contact with the harvested cells (e.g. CellKeep slides) must be treated as biological specimens capable of transmitting infectious agents. Handle and dispose of all hazardous materials according to local regulations.

All work must be conducted in a laboratory at the appropriate containment level using appropriate safety equipment.

Caution: Follow Universal Precautions and local laboratory procedures for personal protection when handling the harvests. Wear the appropriate protective equipment, where necessary (i.e., lab coats, gloves, and eye protection).

Caution: The following may lead to erroneous or inadequate results, and should be avoided:

- Failure to follow procedure
- Failure to store and handle reagents as detailed
- Use of reagents after expiration
- Use of non-calibrated equipment

Caution: Do not use any reagents that become cloudy and appropriately dispose of them immediately.

Consult the manufacturers' MSDS for any chemicals described in the protocol but not provided.

Users must be trained according to applicable regulatory and company/institution requirements. Contact ANGLE for specific training on the use of CellKeep slides

Quality control slides must always be used as detailed in this IFU (refer to Section 7.1).

3. Limitations of the Procedure

For research use only. Not for use in diagnostic procedures. Users must validate with internal procedures for self-directed use.

4. Material Storage and Stability

Store at room temperature (+18°C - +25°C), protected from light and humidity.

Expiration dates are indicated on the label(s) of the individual components.

Note: CellKeep slides and wicking caps are consumable items that are intended for one-time use only.

5. Materials Provided

- CellKeep slide
- Wicking caps
- CellKeep Detacher

6. Materials Required but not Provided

- Deionized H_2O
- Acetone ≥99.9% (should be ice cold (-20°C) prior to use for fixation; place in a -20°C freezer at least one hour before use)
- Foetal Bovine Serum (FBS), filtered and heat inactivated (recommend storing 20 µL aliquots of FBS at -20°C until use)
- 1× Phosphate-Buffered Saline
- 1× Potassium Chloride (KCl) Solution, ~3 M in H_2O
- Blood sample collected into appropriate blood collection tube e.g. Streck Cell-Free DNA BCT or K_2EDTA. Streck Cell-Free DNA BCT containing CTCs
- Heat plate, temperature range: +37°C (± 1°C)
- Fisher Scientific Cytospin™ 4 cytocentrifuge, as configured
- CellKeep Cytospin Adaptor pack (ANGLE product code CLK-ADT-01)
- 1.5 mL Eppendorf tube or equivalent

Materials required but not provided should be used and handled according to the relevant manufacturer's instructions.

7. Procedure

Note: Ensure the ≥99.9% Acetone has been placed in a -20°C freezer at least one hour before use. This will be used for cell fixation; 50 µL per slide will be required.

7.1 Sample harvest and preparation of CellKeep slides

7.1.1 Preparing the drying buffer

- Remove the required volume (20 µL per slide) of sterile, heat inactivated FBS from the fridge/freezer one hour before use, to allow it to reach room temperature (+18°C - +25°C).
- Prepare the drying buffer using the reagents and volumes in Table 3:

Reagents	Qty per slide (µL)	2 slides (µL)	10 slides (µL)
3M KCl	5	10	50
di$H_2$0	175	350	1750
FBS	20	40	200
Total	200	400	2000

Table 3. Preparation of drying buffer.

7.1.2 Preparation of CellKeep slides

- Place ≥99.9% Acetone in a -20°C freezer (this will be used for cell fixation, 50 µL per slide will be required) at least one hour before use.
- Remove the lid of the CellKeep slide funnel.
- For preparation of enriched patient sample or harvested cells:
    - Harvest enriched sample directly from the Parsortix into the CellKeep funnel, allowing the sample to run down the side of the funnel (Figure 1).

Figure 1. Loading the sample into CellKeep funnel

- For preparation of control / mock samples, gently pipette the sample directly into the CellKeep funnel, allowing the sample to run down the side of the funnel (Figure 1).

Note: DO NOT allow the harvest tube or the pipette tip to touch the sample area on the microscope glass slide.

- Screw the lid back onto the CellKeep slide funnel.

Note: When removing and replacing the funnel lid or a wicking cap, hold the base of the funnel flat to prevent premature funnel detachment (Figure 2).

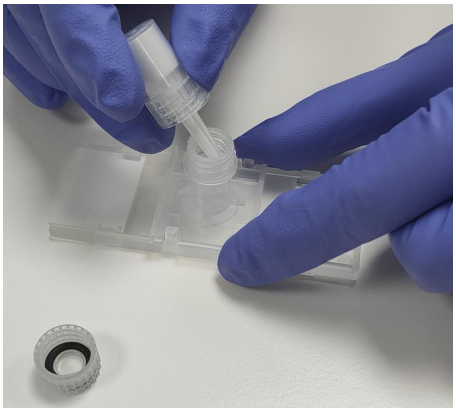

Figure 2. Recommended method for holding the CellKeep slide funnel while screwing the wicking cap / CellKeep slide funnel cap.

- Load the CellKeep slide into a CellKeep Cytospin adaptor. The adaptor has upper and lower brackets that hold the CellKeep slide in place (Figure 3).

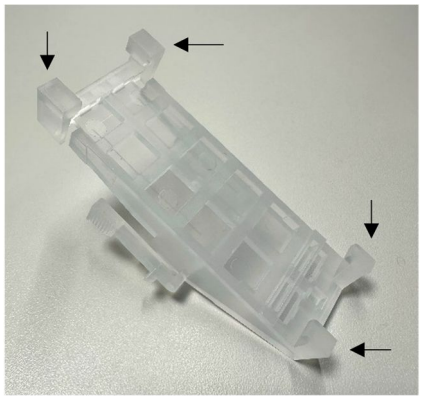

Figure 3. CellKeep Cytospin adaptor brackets.

- Insert the top of the CellKeep slide, annotation tab side first, through the CellKeep Cytospin adaptor upper brackets (Figure 4a).
- Gently push the CellKeep slide flat against the CellKeep Cytospin adaptor and push from the top edge so that the slide is secured beneath the lower brackets (Figure 4b).

Figure 4a. Inserting the CellKeep slide through the CellKeep Cytospin adaptor upper brackets.

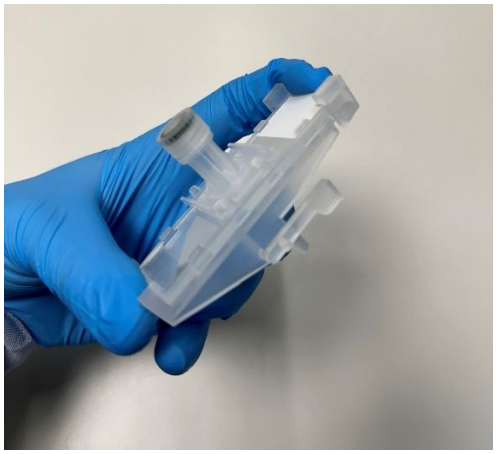

Figure 4b. Securing the CellKeep slide beneath the lower brackets.

- Push in the side arms of the CellKeep Cytospin adaptor and load it into one of the slots in the Cytospin 4 with the funnel facing toward the rotor centre and the lower brackets facing downwards. Align the side arms with the slots in the rotor plate and gently lower the adaptor into the slot. Upon reaching the bottom point, release the side arms to securely lock the adaptor in the slot (Figures 5a – d).

Figure 5a. Approaching the Cytospin 4 with CellKeep Cytospin adaptor side arms secure.

Figure 5b. Loading the CellKeep Cytospin adaptor into the Cytospin 4 slot.

Figure 5c. The CellKeep Cytospin adaptor is locked into the Cytospin 4 slot.

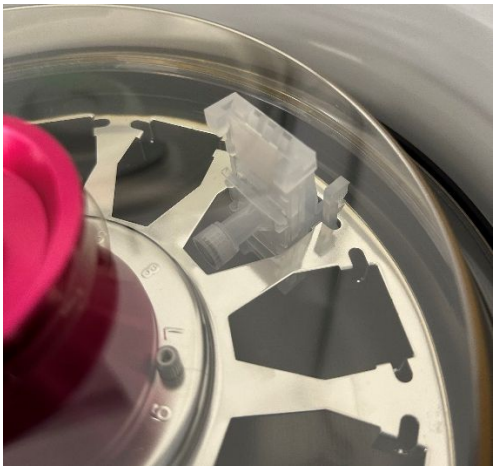

Figure 5d. With internal Cytospin 4 cover in place.

Important: Ensure the Cytospin 4 is appropriately balanced for the number of CellKeep slides that are being centrifuged.

- Set the Cytospin 4 to the following settings for the first step of centrifugation:
  Acceleration: low, Speed: 2000 RPM and Time: 5 minutes.
- Press “Start”.
- Once finished, first remove the CellKeep Cytospin adaptor from the slot by pushing in the side arms to unlock the assembly (reverse the procedure in Figures 5a, b).
- Remove the CellKeep slide from the CellKeep Cytospin adaptor.
- Remove the CellKeep slide funnel cap and screw on a CellKeep wicking cap (Figures 6a, b).

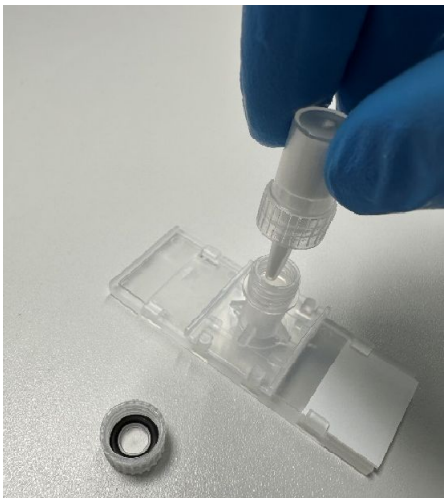

Figure 6a. Screwing on a CellKeep wicking cap.

Figure 6b. CellKeep wicking cap attached.

- Leave the wicking cap for five minutes to draw up the liquid.

Note: Some difference in wicking cap efficiency is expected; time to draw up all liquid may vary.

- After the liquid has all been drawn up, remove the wicking cap and discard.
- Gently pipette 200 µL of drying buffer into the funnel so the buffer runs down the inside wall (Figure 7).

Note: DO NOT pipette directly onto the sample area as this may dislodge cells.

Note: DO NOT touch the sample area with the pipette tip.

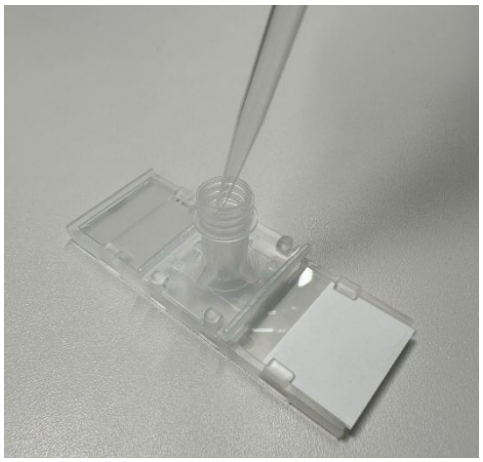

Figure 7. Adding 200 µL of drying buffer after the first centrifugation step.

- Screw the lid back onto the CellKeep slide funnel and load the CellKeep Cytospin adaptor back into the Cytospin 4 (Figures 4a, b and Figures 5a - d).
- Set the Cytospin 4 to the same settings as the first centrifugation step:
  Acceleration: low, Speed: 2000 RPM and Time: 5 minutes.

- Press “Start”.
- Once finished, remove the CellKeep slide from the CellKeep Cytospin adaptor.
- Remove the CellKeep slide funnel lid and screw on a second CellKeep wicking cap (Figures 6a, b).
- Wait for five minutes to draw up the liquid.
- Remove and discard the second wicking cap and screw the lid back onto the CellKeep slide funnel.
- Insert the CellKeep slide into the slide detacher (Figures 8a, b).

Figure 8a. Inserting the CellKeep slide into the slide detacher.

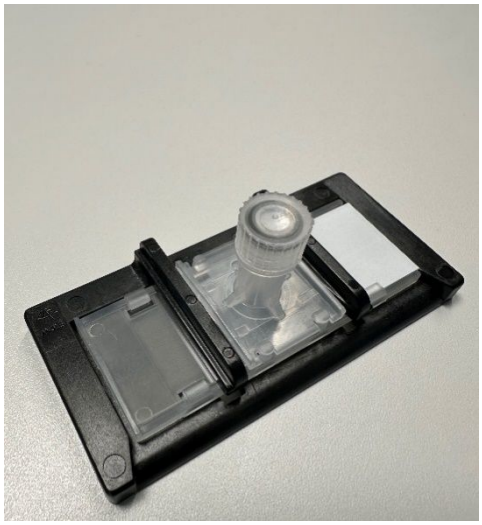

Figure 8b. A CellKeep slide mounted into the slide detacher.

- Remove the funnel from the CellKeep slide by anchoring the slide detacher with one hand and pulling the funnel with the other (Figures 9a, b).

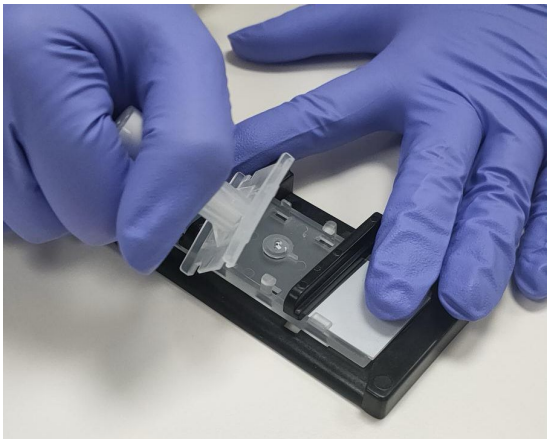

Figure 9a. Removing the funnel from the CellKeep slide.

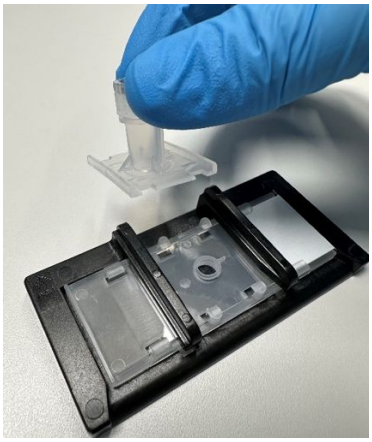

Figure 9b. Detached CellKeep funnel.

- Remove the microscope glass slide from the lower plastic casing (Figures 10a, b).

Note: DO NOT remove the silicon gasket from the slide or touch the sample area.

- Place the microscope glass slide on a heat plate set to 37°C and leave for one hour.

Note: If the heat plate has a lid, DO NOT close it as this will prevent the microscope glass slides from drying.


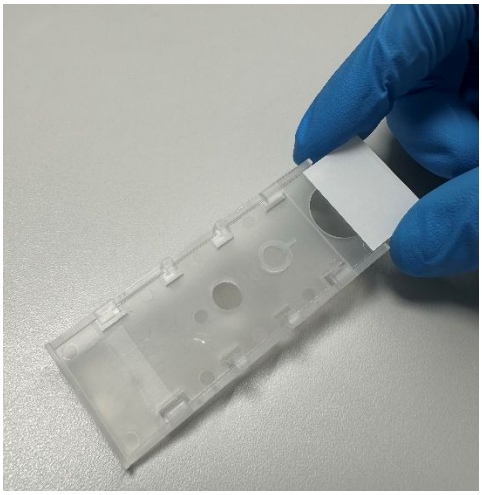

Figure 10a, b. Removing the microscope glass slide from the lower plastic casing.

- Remove the dried microscope glass slide and place on a slide staining tray.
- Pipette 50 µL of ice-cold Acetone drop by drop onto the sample area.
- Place the slides in a freezer (-20°C) for five minutes to fix the cells.
- Remove the slides from the freezer. If there is any residual Acetone on the sample area, gently shake the slide over absorbent paper, then allow the slides to dry at room temperature for approximately 10 minutes.

Note: DO NOT remove the silicon gasket from the slide after fixation unless otherwise specified.

- Slides may now be stored at -20°C for a maximum of one month before staining.

8. Troubleshooting

8.1 Impeded wicking action

Possible cause	**Action**
Improper wicking cap storage	Ensure wicking caps are stored in a cool dry place and not removed from packaging until immediately before use.
Incorrect wicking cap positioning	Ensure wicking cap is screwed all the way onto the funnel assembly, and not cross threaded.
Bubble blocking the wicking cap ingress port	Remove the wicking cap from the funnel and then reattach to clear bubble.

8.2 Overlapping nuclei or uneven cell distribution

Possible cause	**Action**
Improper blood storage	Ensure blood is not stored outside of recommended temperature range (+18 - +25) and/or for shorter or longer than the recommended time.
Improper centrifugation	Ensure slides are handled and prepared as per this IFU.

8.3 Cell morphology degraded

Possible cause	**Action**
Improper blood storage	Ensure blood is not stored outside of recommended temperature range (+18 - +25) and/or for shorter or longer than the recommended time
Incorrect sample fixation	Ensure slides are fixed as described in the IFU with correct reagent, volume, and incubation time/temperature.
Improper centrifugation	Ensure slides are handled and prepared as per this IFU.

8.4 Low or no cells on slide, cells floating on slide

Possible cause	**Action**
Incorrect slide mounting, poor mounting media curing	Ensure slides are mounted using 10 µL of mounting solution and coverslip placed without applying excess pressure. Ensure air bubbles are not trapped and coverslip sealant is applied. Ensure mounting solution is cured before imaging.
Incorrect preparation / application of drying buffer	Ensure drying buffer is prepared with correct concentration of KCl and FBS. Ensure minimal residual drying buffer is left on the slides after wicking.
Incorrect sample fixation	Ensure slides are fixed as described in this IFU with correct reagent, volume and incubation time/temperature.
Improper centrifugation	Ensure slides are prepared as per this IFU.
Harvest not performed correctly	Ensure CTC capture is performed according to the Parsortix instrument IFU. Ensure CTC harvest is performed as per this IFU.
Slides not handled correctly	Ensure sample area is not touched / wiped / scraped at any time. Ensure slides are handled as per this IFU.

9. Technical Support

For customer support including an electronic copy of the Instruction for Use, contact your local distributor or ANGLE technical support (Support@angleplc.com).

10. Definition of symbols

The following symbols may appear on the product labelling:

	Use by date		Contains sufficient for <n> tests
	Batch code		Harmful
	Serial number		Catalogue number
	Caution, consult accompanying documents		Consult Instructions for Use
	Date of Manufacture		Temperature limit
	Manufacturer		Biological hazard
	Keep away from sunlight		Protect from heat and radioactive sources
	Do not reuse		Keep dry

11. Legal Information

ANGLE Europe Limited
10 Nugent Road
Surrey Research Park
Guildford
GU2 7AF
United Kingdom
T: +44 (0)1483 343434
E: support@ANGLEplc.com
www.angleplc.com