

pluriBead[®] Cascade

Cell Separation Protocol

MANUAL



pluriSelect

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Warnings & Limitations

This product is developed for scientific use only. It must not be used for diagnostic or therapeutic purposes in animals or men.

This product is developed for laboratory use only. Users must follow the appropriate laboratory guidelines.

pluriBead® suspension contains conservatives such as sodium azide which are toxic if ingested. Thus, pipetting by mouth must be avoided.

General Advice

Make sure that the kit box is intact and that buffers have not leaked. Do not use a kit that has been damaged.

When working with multiple samples, avoid transferring samples to the wrong mixing containers, strainers or tubes. Ensure that all containers, strainers or tubes are properly labeled using a permanent pen.

Unless otherwise indicated, all steps of this protocol should be carried out at room temperature.

Safety Advice

When handling with biological and chemical materials, always wear a laboratory coat, disposable gloves and protective goggles to prevent infections (e.g. by HIV or hepatitis B viruses) and to avoid injuries. In case of contact between gloves and sample, change gloves immediately.

Storage & Stability

pluriBead® suspension, Wash Buffer, Buffer A (Stabilization Buffer), Buffer B (Incubation Buffer) and Buffer C (Detachment Activation Buffer) should be stored at 2-8°C. Buffer D (Detachment Concentrate) must be frozen at -20°C.

Properly stored and handled, pluriBead® suspension and buffers are stable until the expiration date stated on the label. Deviant storage conditions will lead to poorer separation results.

Thoroughly resuspend pluriBead® suspension before use. Avoid bacterial or fungal contamination of the pluriBead® suspension and provided buffers.

Preventing Cell Stress & Contamination

When pipetting the sample into the mixing container, avoid the contamination of the containers rim.

Always change pipette tips between liquid transfers. Use filter pipette tips.

Avoid touching the mesh of the strainer with the pipette tip.

Avoid any unnecessary contact with the inner sterile surfaces of the kit components. In particular, the surfaces of the mixing container and the strainer should remain sterile to avoid cross contamination.

pluriBead® Technology

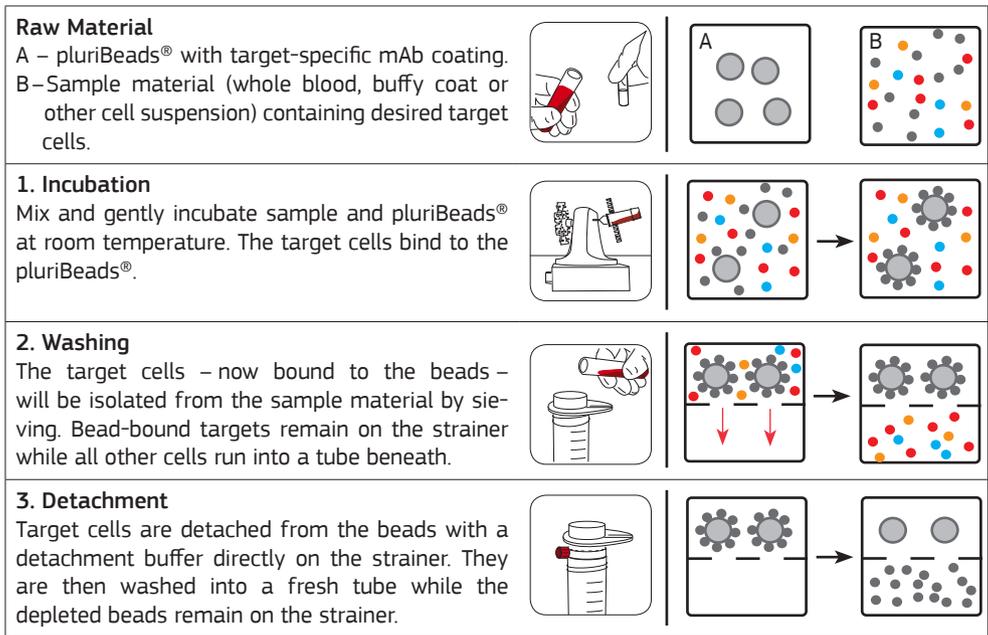
pluriBead® uses non-magnetic monodispersed microparticles (beads) for the separation of cell mixtures. Their surface is coated with monoclonal antibodies (mAb) directed against specific structures on the target cell surface.

During incubation, the target cells in suspension will bind to the pluriBeads® and can be separated afterwards by a pluriStrainer® (size exclusion) from the suspension. The beads are larger than the cells and thus cannot be phagocytized by them.

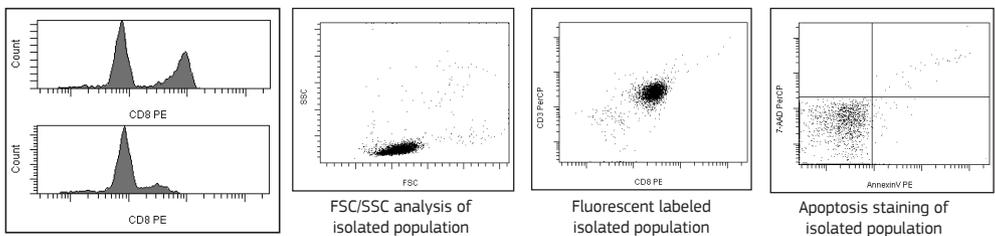
Pretreatment of the blood (e.g. density centrifugation, erythrolysis or other target concentration) is not required.

pluriBead® should be used for research use only.

pluriBead® Principle



Typical pluriBead® Cell Separation Profile: Example CD8



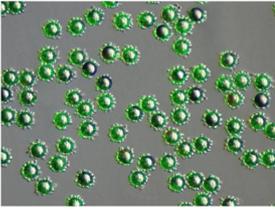
Histogram of whole blood before (top) and after (below) depletion

FSC/SSC analysis of isolated population

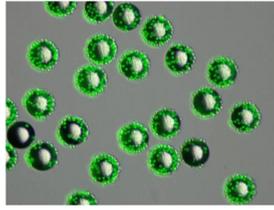
Fluorescent labeled isolated population

Apoptosis staining of isolated population

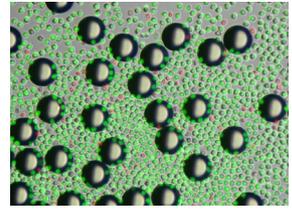
pluriBead® Particles - Phase Contrast



S-pluriBead®



M-pluriBead®



Cells after detachment

Living cells stained with Calcein AM (green)
Dead cells stained with Propidium Iodide (red)

pluriBead® Size

| Specification | S-pluriBead® | M-pluriBead® |
|---|--|--|
| Illustration | <p>A - target cell</p> | <p>A - target cell</p> |
| pluriBead® Size | 32 µm | 65 µm |
| Maximum isolated cells per separation | 1x 10 ⁷ | 5x 10 ⁷ |
| Maximum bead suspension per pluriStrainer | 400 µl | 1,000 µl |
| Recommended application | medium number ($\leq 2 \times 10^6$) of targets, rare cells, and circulating tumor cells (CTC) | large number of targets in a sample (e. g. buffy coat) |
| Sample material | whole blood, tissue, PBMC, cell culture, buffy coat, liquor | buffy coat, whole blood*, tissue, PBMC, cell culture * only recommended for cells with high concentration (e.g. granulocytes) |
| pluriBead® material | Polystyrene | Polystyrene |
| Minimum sample volume | 200 µl | 500 µl |

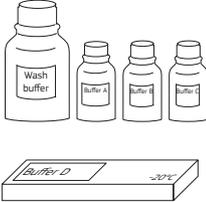
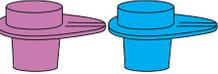
If you have problems choosing the right pluriBead® size use the interactive selection guide

<http://pluriselect.com/selection-guide.html>

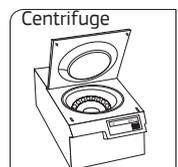
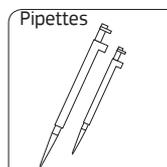
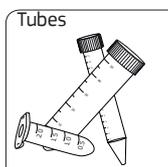
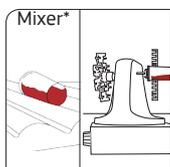
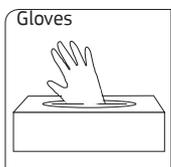
pluriBead® Suspension

| pluriBeads | Picture | Description | Storage Conditions |
|--|---|--|--------------------|
| S-pluriBead® suspension - purple cap M-pluriBead® suspension - blue cap |  | Catcher particles labeled with specific antibodies | Store at 4–8°C |

S-pluriBead® Reagent Kit & M-pluriBead® Reagent Kit

| Kit Components | Picture | Description | Storage Conditions |
|---|---|--|--|
| Wash buffer (10x Stock Solution) |  | Buffer for washing steps. Dilute before use. | Store at 4–8°C |
| Buffer A (Stabilization Buffer) | | Chelating agent for preserving blood sample | Store at 4–8°C |
| Buffer B (Incubation Buffer) | | Buffer to increase density of sample esp. for PBMC isolation | Store at 4–8°C |
| Buffer C (Detachment Activation Buffer) | | Add 200 µl to detachment concentrate vial for its activation | Store at 4–8°C |
| Buffer D (Detachment Concentrate) |  | Detachment of target from pluriBeads | Store at –20°C  |
| S-pluriStrainer® S / 30 µm (purple, max. load: 400 µl S-pluriBead® suspension) M-pluriStrainer® S / 60 µm (blue, max. load: 1000 µl M-pluriBead® suspension) |  | Strainer for separating pluriBeads with target cells from sample and for sample pre-filtration | Room Temperature |
| Connector Ring incl. Luer-Lock |  | Connection to 50 ml tube. Essential for detaching the pluriBeads® | Room Temperature |
| Funnel |  | Supports sample load onto pluriStrainer® | Room Temperature |

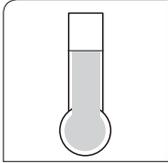
Additional required materials for working with pluriBead®



*No orbital shakers or

1. Sample Preparation and Target Binding

1.1 Preparation of Buffers (before the start of the separation)



Room temperature

- Bring all provided reagents to room temperature.
(For the detachment concentrate: Do not use a 37°C water bath or incubator)
- **Dilute** 10x **Wash Buffer** (sufficient for 1,000 ml Wash Buffer) with sterile high-purity water (E.g. dilute 100 ml 10x Wash Buffer with 900 ml steril water)
- Add 200 µl **Buffer C** into 1 vial of **Buffer D** (1.8 ml).



1.2 Preparation of Sample Material



| Whole Blood | Tissue/PBMC | Buffy Coat |
|---|---|---|
| <ul style="list-style-type: none"> • Add 50 µl of provided Buffer A per 1 ml sample. • CD14: Before separating CD14+ cells from a sample, remove sCD14! (see p. 11) • Mouse whole blood: Pre-filter sample to remove aggregates. | <ul style="list-style-type: none"> • Prepare a single cell suspension. <i>Take up the cell pellet in 500 µl Buffer B and 500 µl Wash Buffer.</i> <i>Adjust the concentration of cells at max. 5x10⁶ targets per 1 ml buffer.</i> • Pre-filter sample with S-pluriStrainer® to remove aggregates. | <ul style="list-style-type: none"> • Add 50 µl of provided Buffer A per 1 ml sample. • Pre-filter buffy coat. <p><i>Attach a provided strainer to a sterile 50 ml centrifuge tube. Place a provided funnel on top. Carefully pour your sample into the funnel.</i></p> <p><i>If the buffy coat clogs the strainer, prepare first PBMC or use another buffy coat!</i></p> <ul style="list-style-type: none"> • Alternatively use Buffy Coat Add-On (Order.No.: 01-00600-10) |

1.3 Binding of Targets



- Thoroughly **resuspend pluriBead®** suspension by vortexing the tube.
- Add **S-pluriBead®** suspension, **M-pluriBead®** and sample into a sterile mixing tube:

| | |
|-----------------------|---|
| Whole Blood: | 40 µl S-pluriBead® suspension per 1 ml 100 µl M-pluriBead® suspension per 1 ml |
| Other samples: | 40 µl S-pluriBead® suspension per 1x10⁶ targets 100 µl M-pluriBead® suspension per 1x10⁶ targets |

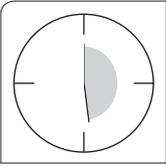
Recommended tube sizes:

Sample + pluriBeads® 0.2–2 ml: 2 ml tube (**round bottom**)

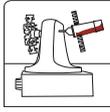
Sample + pluriBeads® 2–6 ml: 15 ml tube or use the blood collection tube

Sample + pluriBeads® 6–50 ml: 50 ml tube



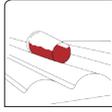


· **Incubate sample** up to 30 min. pluriBead® must remain in suspension.

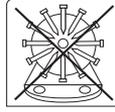


pluriPlix®
(by pluriSelect)

10–15 rpm/7.5° angle 10–15 rpm



Horizontal roller mixer
(with tilting function)



Rotator mixer



Laboratory rocker

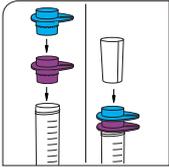


Orbital shaker

For optimal results, it is important that the sample material is mixed gently! Since the particles are larger than the cells, the particles must remain in suspension during the incubation period and may not settle, so that the cells can bind to the particles.

While incubating the sample, unfreeze the detachment buffer according to the amount of your sample material and pre-warm the washing buffer to room temperature. Do not use a 37°C water bath or an incubator.

2. Washing



- Attach a **S-pluriStrainer®** to a sterile **50 ml** centrifuge **tube**.
The bigger opening of the strainer must be on the top.
- Attach an **M-pluriStrainer®** onto **S-pluriStrainer®**.
- **Optional for sample more than 4ml:** Place the supplied **funnel** on top of the strainers and maximum application volume of liquid on the strainer increases to 15 ml.
- Add 1 ml Wash Buffer (diluted, see step 1.1) to equilibrate the strainer and support separation.

Use one tube and strainer stack for each:

- max 160 µl **S-pluriBead®** - or - max 400 µl **M-pluriBead®** used in sample



- **Pour sample-bead-suspension** onto the **M-pluriStrainer®**.

Unbound cells run through the strainers into the centrifuge tube, the bead-bound target cells remain on the strainers.

The flow-through can be used for further cell isolation.



- Recommendation: Use a 5 ml pipette (no serological pipette).
- **Wash** off the bead-sample traces from the **funnel** in 2 ml steps* and discard the funnel.
- **Wash the top strainer** sufficiently in 2 ml steps with Wash Buffer.
- Wash in a **circular motion**, avoid washing the middle of the strainer only.



- Take off the **M-pluriStrainer®** and attach it to a sterile 50 ml centrifuge tube. Then, wash both strainers with 5 x 2 ml wash buffer each. Do work fast enough to prevent the cells on both sieves from drying out.

Watch out the tubes beneath the strainers contains no more than 45 ml of sample/Wash Buffer! Use several tubes if necessary.

The bead-bound target cells on the strainer are now ready for further use.

*Note: Wash until you do not see any red spots any more, plus 3 additional washing steps.



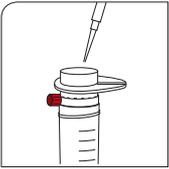
After washing, erythrocytes or other unwanted material

might still adhere to the inner surface of the strainer. To avoid target contamination with these during later steps of the protocol, you can also wash the inner and outer surface as well as the bottom of the strainer.

When adding Wash Buffer with too much pressure, targets on the strainer might be splashed away.

Notice

Target lysis for protein or DNA/RNA analysis

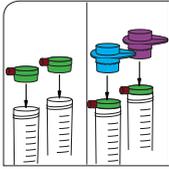


For rapid and consistent results in protein or gene expression analysis, lyse the targets while they are still attached to the beads.

- Attach the connector tightly to a new 50 ml centrifuge tube.
- Close the Luer-Lock and attach the strainer with the isolated target cells.
Caution: Do not tilt the strainer!
- Add lysis buffer according to the manufacturers direction.

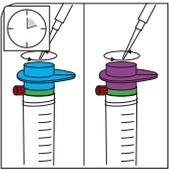
3. Detachment

(Target cells will be released from the pluriBeads into the tube.)

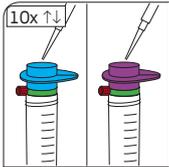


- **Attach two Connector Rings** straight and tight to two new 50 ml tubes.
- **Attach and close the Luer-Locks.**
- **Attach both strainers** with the isolated target cells to the Connector Rings. Caution: Fit even and do not tilt the strainers!
- **Add 1 ml Wash Buffer** along the wall of the strainer.

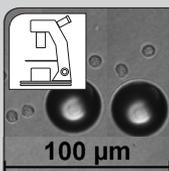
- Optional check 1** – Check if the target cells are bound to the beads.
- Resuspend the beads by gently moving the strainer in a circular way.
 - Retain a small amount of sample (10 μ l) and place it on a microscope slide or in a microwell plate.
 - Check if the target cells are bound to the beads.



- **Add 1 ml activated Buffer D** (200 μ l Buffer C into 1 vial Buffer D) along the wall of each strainer.
- Swirl the sample gently and **incubate** it for **10 min** at room temperature. Gently move each strainer in a circular way every 2 min. **Do not pipette!** If liquid drops through the strainer, check whether the Luer-Lock is closed and the strainer is placed correctly. It is no problem though if 100–200 μ l of liquid run through the strainer during the 10 min detachment time.



- After incubation, add **1 ml Wash Buffer on each strainer.**
- Separate the cells from the beads by **pipetting up and down** the sample on each strainer with a 1 ml pipette (10 times). Avoid air bubbles and do not touch the mesh with the pipette.



- Optional check 2** – Check if the cells are released from the beads.
- Retain a small amount of sample (10 μ l) and place it on a microscope slide or in a microwell plate.
 - Check under the microscope if the cells are released from the beads.
 - If the beads are still rosetted with cells, extend incubation time for another 5 min and repeat pipetting up/down the sample on the strainer (10x).



- **Open Luer-Lock.** The detached cells now run into the 50 ml centrifuge tube, pluriBeads® remain on each pluriStrainer®.
- **Wash each strainer 10 times** with 1 ml Wash Buffer or cell culture medium. Wait until buffer has drained off, before adding new buffer.
- **Remove connectors and strainers** from the tube by lifting the connector with the thumb. Discard both connector and strainer.



- For **centrifugation**, **pour** the suspension with the detached cells **into a fresh 15 ml tube.** (Larger tubes will lose approx. 30% of your targets!)
- Centrifuge the cells **10 min at 300 x g without brake.**
- Carefully remove the supernatant with a **pipette down to 500 μ l.** (Pouring off the supernatant will lose approx. 20% of your targets!)
- Transfer the cells into Wash Buffer or medium of your choice. The cells are now separated from the beads and can be used for further experiments.

Common Error Sources

Target Yield 40 μ l S-pluriBead[®] suspension can bind up to 1×10^6 target cells. This depends on the existing amount of target cells in the sample material, the density of receptors on target cells and an optimal mixing of the pluriBeads[®] with the sample.

The initial concentration of leukocytes in whole blood can be determined by using a hemocytometer or by hand with a counting chamber and Turk's solution.

Monocytes from Whole Blood When separating CD14 positive cells from a sample, remove sCD14 first!

1. Dilute whole blood with Wash Buffer (1 ml whole blood + 2 ml Wash Buffer).
2. Centrifuge 10 min at 300 x g (no brake).
3. Detach plasma and supernatant to approx. 1 cm above the blood.
Do not pipette too closely to the buffy coat layer to avoid pipetting cells!
4. Repeat step 1 to 3.

Use the **concentrated** cells for the separation of CD14+ cells but calculate the pluriBead[®] volume according to the starting sample volume.

Tissue/PBMC Detach adherent cells from the tube surface, or rather isolate tissue cells, very gentle. Thereto, keep the digestion time as short as possible. Long reaction times damage the cell surface receptors and thus reduce the efficiency of pluriBead[®].

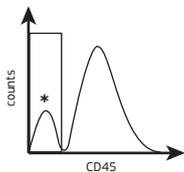
Stop digestion by adding complete medium and separate the cells from one another by pipetting. Afterwards, transfer the cells into a centrifuge tube and pelletize the cells.

To avoid cell aggregations, you can attach a strainer to the centrifuge tube. While the single cells pass through, cell aggregations are held back by the strainer.

Detachment When adding the activated Buffer D to your bound targets, take care to not pour buffer into the middle of the strainer. Rather pour it onto the inner rim of the strainer so that the sample is swirled.

When separating the cells from the beads, do pipette carefully but not too cautiously. Rude pipetting will result in air exchange and dripping of the buffers. Too cautious pipetting, however, will not separate all targets from the catcher particles. As a result, your yield will be low.

Purity Analysis For cell analysis always use CD45 for the staining of Leucocytes isolated from whole blood, buffy coat or PBMC. That will help to exclude unwanted erythrocytes (RBC), platelets and debris for the analysis.



* Platelets, red blood cells and debris.

All buffers and consumables can also be ordered individually from www.pluriselect.com.

Buffers

| Product | Order No. | Size |
|--|-------------|-------------|
| Buffer A (Stabilization Buffer) | 60-00070-12 | 10 ml |
| Buffer B (Incubation Buffer) | 60-00060-12 | 10 ml |
| Buffer C (Detachment Activation Buffer) | 60-00045-12 | 10 ml |
| Buffer D (Detachment Concentrate) | 60-00040-12 | 10 x 1.8 ml |
| Wash buffer (10x Stock Solution) | 60-00080-10 | 100 ml |

Consumables

| Product | Order No. | Size |
|-------------------|-------------|---------|
| S-pluriStrainer® | 43-50030-03 | 25 pcs. |
| M-pluriStrainer® | 43-50060-03 | 25 pcs. |
| Connector Ring | 41-50000-03 | 25 pcs |
| Funnel | 42-50000-03 | 25 pcs |
| Buffy Coat Add-On | 01-00600-10 | 1 Kit |

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Additional Support:
<http://pluriselect.com/video-tutorials.html>
support@pluriselect.com

Manual_pluriBead_Cascade_EN_V5-7