pluriBead® KIT

Cell Separation Protocol

S-Bead
2 Contents

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pluriBead® Kit Components

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>Picture</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pluriBead® suspension (S-pluriBeads® white cap)</td>
<td></td>
<td>pluriBeads® with antibodies, Store at 4–8°C</td>
</tr>
<tr>
<td>Wash buffer</td>
<td></td>
<td>Store at 4–8°C</td>
</tr>
<tr>
<td>Incubation buffer</td>
<td></td>
<td>Store at 4–8°C</td>
</tr>
<tr>
<td>Stabilization buffer</td>
<td></td>
<td>Store at 4–8°C</td>
</tr>
<tr>
<td>Detachment buffer</td>
<td></td>
<td>Store at –20°C</td>
</tr>
<tr>
<td>Strainer (S-pluriStrainer® purple, max. load: 400 µl S-pluriBead® suspension)</td>
<td></td>
<td>Strainer for sample pre-filtration and for separating target cells from sample, Store at room temperature</td>
</tr>
<tr>
<td>Connector incl. Luer-Lock</td>
<td></td>
<td>Connection to 50 ml tube, Store at room temperature</td>
</tr>
<tr>
<td>Funnel</td>
<td></td>
<td>Supports sample load onto pluriStrainer®, Store at room temperature</td>
</tr>
</tbody>
</table>

Additional Materials for working with pluriBead®

- Gloves
- Mixer*
- Tubes
- Pipettes
- Centrifuge

*No orbital shakers or laboratory rockers!
**pluriBead® Technology**

pluriBead® uses non-magnetic monodispersed microparticles (beads) for the sorting of cell mixtures. The beads are larger than the cells and thus cannot be phagocytized by them. Their surface is modified with monoclonal antibodies that recognize 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.

Pretreatment of the blood, such as the production of a mononuclear cell fraction (PBMC – Peripheral Blood Mononuclear Cells) or others (e.g. erythroylisis or target concentration), is not required.

pluriBead® has been developed for research use only.

**pluriBead® Principle**

**Raw Material**

A – pluriBeads® with target-specific surface coating.
B – Sample material (whole blood, buffy coat or other cell suspension) with desired targets.

1. **Binding**
Sample and pluriBeads® are mixed and gently incubated at room temperature. The target cells bind to the pluriBeads®.

2. **Washing**
The target cells – now bound to the beads – are isolated from the sample material by filtration. Bead-bound targets remain on the strainer while all other cells run into a tube beneath.

3. **Detachment**
Target cells are detached from the beads with a detachment buffer directly on the strainer. They are then washed into a fresh tube while the depleted beads remain on the strainer.

**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
0. Coupling of Antibodies to Universal pluriBeads®*

0.1 Linking with External Antibody

- Use pluriBeads®, your own antibody and PBS solution at room temperature.
- Mix reagents in a 1.5 ml reaction tube.

<table>
<thead>
<tr>
<th>S-pluriBead® suspension</th>
<th>400 µl (1x10⁶ beads for 1x10⁷ targets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Volume antibody</td>
<td>Minimum 10 µg antibody</td>
</tr>
<tr>
<td>+ Volume PBS</td>
<td>Maximum 90 µl PBS</td>
</tr>
<tr>
<td>= Total volume</td>
<td>= 500 µl</td>
</tr>
</tbody>
</table>

- Incubate the sample 3–4 h at room temperature. pluriBeads® must remain in suspension.

0.2 Washing

- Centrifuge the suspension and remove supernatant.
  1. Centrifuge for 2 min at 5,000 x g without brake (if possible).
  2. Carefully remove ~350 µl supernatant.
  3. Add 1.2 ml PBS solution into the reaction tube to obtain a total volume of 1.5 ml.
  4. Vortex suspension shortly.
  5. Repeat steps 1 to 4 (3x), removing 1.2 ml supernatant in step 2.

0.3 Usage

- Immediate use: Add 350 µl PBS solution (pH 7.4) onto the pellet.
- Long-term storage (6 months at 4°C): Add 350 µl PBS solution (pH 7.4) with 0.05% sodium azide and 0.1% BSA onto the pellet.

Both ways, you obtain ~400 µl suspension with labeled catcher particles (~1x10⁶ beads).

*Universal pluriBeads® can be coupled with any external antibody and subsequently can be applied according to the standard pluriBead® protocol (see pp. 5–8). Efficiencies of externally labeled pluriBeads® may vary depending on the antibody employed.
1. Sample Preparation and Target Binding

1.1 Preparation of Buffers

- Bring all provided reagents to room temperature. (For the detachment buffer: Do not use a 37°C water bath or incubator! Rather use incubation period to this end.)
- Dilute provided 10x wash buffer with sterile high-purity water. Check pH value. If necessary, adjust it to pH 7.4.
- Carry out all separation steps at room temperature.

1.2 Preparation of Sample Material

<table>
<thead>
<tr>
<th>Whole Blood</th>
<th>Tissue/PBMC</th>
<th>Buffy Coat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Add</strong> provided stabilization buffer: 50 µl per 1 ml sample.</td>
<td><strong>Prepare a single cell suspension.</strong></td>
<td><strong>Add</strong> provided stabilization buffer: 50 µl per 1 ml sample.</td>
</tr>
<tr>
<td>CD14: Before separating CD14+ cells from a sample, remove sCD14! (see p. 9)</td>
<td><strong>Pre-filter</strong> sample to remove aggregates.</td>
<td><strong>Pre-filter</strong> buffy coat.</td>
</tr>
<tr>
<td>Mouse whole blood: <strong>Pre-filter</strong> sample to remove aggregates.</td>
<td><strong>Adjust targets with provided incubation and wash buffer.</strong></td>
<td>Attach a provided strainer to a sterile 50 ml centrifuge tube. Place a provided funnel on top. Carefully pour your sample into the funnel. If the buffy coat clogs the strainer, use a different sample!</td>
</tr>
</tbody>
</table>

1.3 Binding of Targets

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

| Whole Blood: 40 µl S-pluriBead® suspension per 1 ml | Other samples: 40 µl S-pluriBead® suspension per 1x10⁶ targets | Alternatively: 40 µl S-pluriBead® suspension per 1x10⁶ total cells |

Recommended tube sizes:
- Sample + pluriBeads® 0.2–2 ml: 2 ml tube (round bottom)
- Sample + pluriBeads® 2–6 ml: 15 ml tube
- Sample + pluriBeads® 6–50 ml: 50 ml tube

- Incubate sample up to 30 min. pluriBeads® must remain in suspension.

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

| Whole Blood: 40 µl S-pluriBead® suspension per 1 ml | Other samples: 40 µl S-pluriBead® suspension per 1x10⁶ targets | Alternatively: 40 µl S-pluriBead® suspension per 1x10⁶ total cells |

Recommended tube sizes:
- Sample + pluriBeads® 0.2–2 ml: 2 ml tube (round bottom)
- Sample + pluriBeads® 2–6 ml: 15 ml tube
- Sample + pluriBeads® 6–50 ml: 50 ml tube

- Incubate sample up to 30 min. pluriBeads® must remain in suspension.
2. Washing

2. Volume-dependent Washing (Consider applied volumes of sample and beads!)

<table>
<thead>
<tr>
<th>Sample volume &lt;4 ml, S-pluriBead® suspension &lt;400 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small Sample</strong></td>
</tr>
<tr>
<td>• Attach an S-pluriStrainer® to a sterile 50 ml centrifuge tube. The bars of the strainer must be on the top.</td>
</tr>
<tr>
<td>• Add 1 ml wash buffer to equilibrate the strainer and support separation.</td>
</tr>
<tr>
<td>• Predilute sample material &lt;2 ml with 2 ml wash buffer before filtering.</td>
</tr>
<tr>
<td>• Pour sample (up to 4 ml) directly onto the strainer.</td>
</tr>
<tr>
<td>Unlabeled cells run through the strainer into the centrifuge tube, the rosetted beads with target cells remain on the strainer. The flow-through can be used for further cell isolation.</td>
</tr>
<tr>
<td>• Recommendation: Use a 5 ml pipette (no serological pipette).</td>
</tr>
<tr>
<td>• Wash the strainer sufficiently in 2 ml steps*.</td>
</tr>
<tr>
<td>• Wash in a circular motion, avoid washing the middle of the strainer only.</td>
</tr>
<tr>
<td>The bead-bound target cells on the strainer are now ready for further use.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample volume &gt;4 ml, S-pluriBead® suspension &lt;=400 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medium Sample</strong></td>
</tr>
<tr>
<td>• Attach an S-pluriStrainer® to a sterile 50 ml centrifuge tube. The bars of the strainer must be on the top.</td>
</tr>
<tr>
<td>• Place the supplied funnel on top of the strainer. Maximum application volume of liquid on the strainer increases to 15 ml.</td>
</tr>
<tr>
<td>• Add 1 ml wash buffer to equilibrate the strainer and support separation.</td>
</tr>
<tr>
<td>• Pour sample (more than 4 ml) into the funnel.</td>
</tr>
<tr>
<td>Unlabeled cells run through the strainer into the centrifuge tube, the rosetted beads with target cells remain on the strainer. The flow-through can be used for further cell isolation.</td>
</tr>
<tr>
<td>• Recommendation: Use a 5 ml pipette (no serological pipette).</td>
</tr>
<tr>
<td>• Wash off the bead-sample traces from the funnel in 2 ml steps* and discard the funnel.</td>
</tr>
<tr>
<td>• Wash the strainer sufficiently in 2 ml steps*.</td>
</tr>
<tr>
<td>• Wash in a circular motion, avoid washing the middle of the strainer only. Watch out that the tube beneath the strainer contains no more than 45 ml of sample/wash buffer! Use several tubes if necessary.</td>
</tr>
<tr>
<td>The bead-bound target cells on the strainer are now ready for further use.</td>
</tr>
</tbody>
</table>
2. Washing

Large Sample

Sample volume >4 ml, S-pluriBead® suspension >400 µl

- According to the applied amount of pluriBead® suspension, use several S-pluriStrainers® (see table below).

Attach each strainer to a sterile 50 ml centrifuge tube. The bars of the strainers must be on top.

<table>
<thead>
<tr>
<th>Total volume of S-pluriBead® suspension in your sample</th>
<th>400 µl</th>
<th>800 µl</th>
<th>1.2 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of strainers and tubes to be applied</td>
<td>1x</td>
<td>2x</td>
<td>3x</td>
</tr>
</tbody>
</table>

- Place the supplied funnels on top of the strainers.

Maximum application volume of liquid on the strainer increases to 15 ml.

- Add 1 ml wash buffer each to equilibrate the strainers and to support separation.

- Divide the sample according to the number of funnels and carefully pour the sample into them.

Unlabeled cells run through the strainer into the centrifuge tube, the rosetted beads with target cells remain on the strainer.

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 funnels in 2 ml steps* and discard the funnels.

- Wash the strainers sufficiently in 2 ml steps*.

- Wash in a circular motion, avoid washing the middle of the strainers only. **Watch out that the tubes beneath the strainers contain no more than 45 ml of sample/wash buffer! Use several tubes if necessary.**

The bead-bound target cells on the strainers are now ready for further use. The samples can be merged after detachment.

Notice

Target lysis for 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 the provided 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 declaration.

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

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

- **Attach the connector** straight and tight to the provided 50 ml tube.
- **Close the Luer-Lock.**
- **Attach the strainer** with the isolated target cells to the connector ring.
  
  Caution: Do not fit too tight, do not tilt the strainer!
- **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 μl) 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 detachment buffer** along the wall of the strainer.
- Swirl the sample gently and **incubate** it for **10 min** at room temperature. Gently move the 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 µl of liquid run through the strainer during the 10 min detachment time.

- After incubation, add another **1 ml wash buffer**.
- Separate the cells from the beads by **pipetting up and down** the sample on the strainer with a 1 ml pipette (10x).
  Avoid air bubbles and do not touch the strainer with the pipette.

**Optional check 2 – Check if the cells are released from the beads.**

- Retain a small amount of sample (10 μl) 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, stretch incubation time for another 5 min and repeat pipetting up/down the sample on the strainer (10x).

- **Open the Luer-Lock.** The detached cells now run into the centrifuge tube.
- **Wash the strainer** with 10x1 ml wash buffer or cell culture medium.
  Wait until buffer has drained off, before adding new buffer.
- **Remove connector and strainer** 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 μl.**
  (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.
## Troubleshooting

### Common Error Sources

| Target Yield | 40 µl S-pluriBead® suspension can catch up to $1 \times 10^6$ 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 particles in 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. |
| Whole Blood | When separating CD14 positive cells from a sample, remove sCD14 first! 1. Dilute wholeblood with wash buffer (1 ml wholeblood + 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 this procedure once again. 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 reaction time as short as possible. Long reaction times damage the cell surface receptors and thus reduce the efficiency of pluriBead®. Stop the reaction 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. |
| Washing | After washing pluriStrainer®, 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. **Note:** When adding wash buffer with too much pressure, targets on the strainer might be splashed away. |
| Detachment | When adding the detachment buffer 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. Cautious pipetting, however, will not separate all targets from the catcher particles. As a result, your yield will be low. |
Extra Washing Buffer
Prepare a solution containing phosphate-buffered saline (PBS) pH 7.4, 0.5% bovine serum albumin (BSA) and 2 mM EDTA.
Filter buffer sterile.
Store buffer at 4–8°C.

Extra PBS
For 1 l of 1x PBS, prepare a solution as follows:
1. Start with 800 ml of distilled water.
2. Add 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na$_2$HPO$_4$ and 0.24 g of KH$_2$PO$_4$.
3. Adjust the pH to 7.4 with HCl.
4. Add distilled water to a total volume of 1 litre.

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

<table>
<thead>
<tr>
<th>Buffers</th>
<th>Order No.</th>
<th>Consumables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product</td>
<td></td>
<td>Product</td>
</tr>
<tr>
<td>Detachment buffer</td>
<td>60-00040-12 (20 ml)</td>
<td>S-pluriStrainer®</td>
</tr>
<tr>
<td>Incubation buffer</td>
<td>60-00060-12 (50 ml)</td>
<td>Connecting ring</td>
</tr>
<tr>
<td>Stabilization buffer</td>
<td>60-00070-12 (25 ml)</td>
<td>Funnel</td>
</tr>
<tr>
<td>Wash buffer (10x)</td>
<td>60-00080-10 (100 ml)</td>
<td>Buffy Coat Add-On</td>
</tr>
</tbody>
</table>

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Additional Support:
http://pluriselect.com/video-tutorials.html
support@pluriselect.com
Skype ID: pluriselect

V5-1_general manual_s-bead