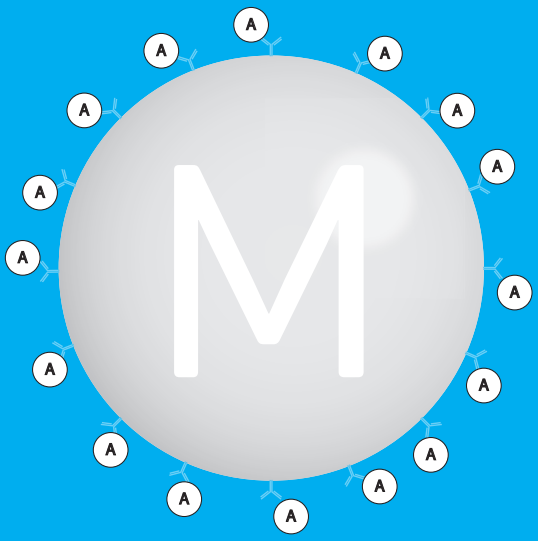
# M-pluriBead®

## *Cell Separation Protocol*

MANUAL




A - Target cells

pluriSelect

support@pluriselect.com | www.pluriselect.com

New Detachment Protocol

## 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. densitiy centrifugation, erythrolysis or other target concentration) is not required.

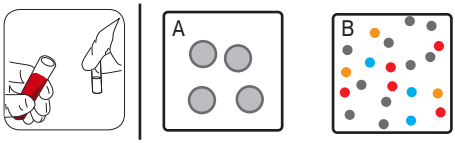
pluriBead® should be used for research use only.

## pluriBead® Principle

**Raw Material**

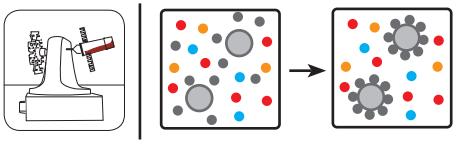
A – pluriBeads® with target-specific mAb coating.

B – Sample material (whole blood, buffy coat or other cell suspension) containing desired target cells.



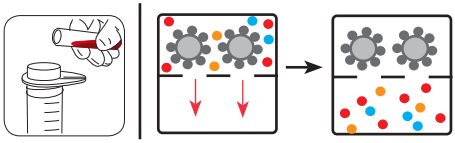

**1. Incubation**

Mix and gently incubate sample and pluriBeads® at room temperature. The target cells bind to the pluriBeads®.



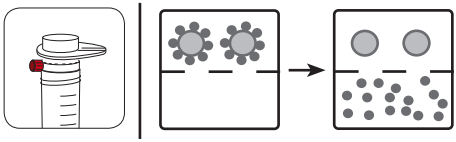
**2. Washing**

The target cells – now bound to the beads – will be isolated from the sample material by sieving. Bead-bound targets remain on the strainer while all other cells run into a tube beneath.

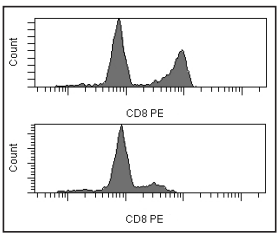


**3. Detachment**

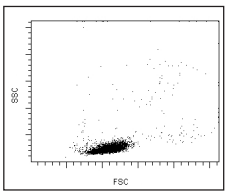
Target cells are detached from the beads with a detachment buffer directly on the strainer. They are then washed into a fresh tube while the depleted beads remain on the strainer.



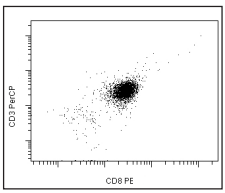
## Typical pluriBead® Cell Separation Profile: Example CD8



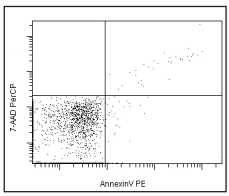

Histogram of whole blood before (top) and after (below) depletion




FSC/SSC analysis of isolated population



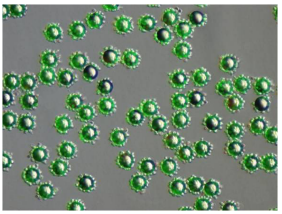

Fluorescent labeled isolated population



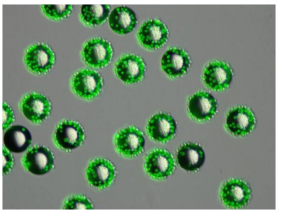

Apoptosis staining of isolated population

# pluriBead® in Detail

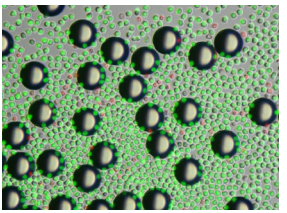
## pluriBead® Particles - Phase Contrast



S-pluriBead®



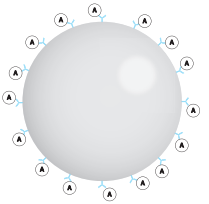
M-pluriBead®



Cells after detachment

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

## pluriBead® Size

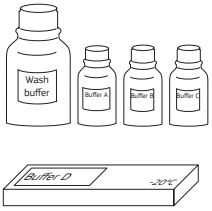
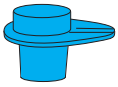
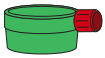
| Specification | S-pluriBead® | M-pluriBead® |
|---|---|---|
| Illustration |  A - target cell |  A - target cell |
| pluriBead® Size | 32 µm | 65 µm |
| Maximum isolated cells per separation | 1x $10^7$ | 5x $10^7$ |
| Maximum bead suspension per pluriStrainer | 400 µl | 1,000 µl |
| Recommended application | medium number (≤2x$10^6$) 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 |
|---|---|---|---|
| pluriBead suspension<br>(M-pluriBeads - blue cap) |  | Catcher particles labeled with specific antibodies | Store at 4-8°C |

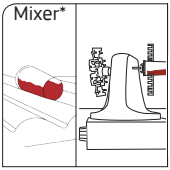
## pluriBead® Reagent Kit

| Kit Components | Picture | Description | Storage Conditions |
|---|---|---|---|
| Wash Buffer<br>(10x Stock Solution) |  | Buffer for washing steps. Dilute before use. | Store at 4–8°C |
| Buffer A<br>(Stabilization Buffer) | | Chelating agent for preserving blood sample | Store at 4–8°C |
| Buffer B<br>(Incubation Buffer) | | Buffer to increase density of sample esp. for PBMC isolation | Store at 4–8°C |
| Buffer C<br>(Detachment Activation Buffer) | | Add 200 µl to Detachment Concentrate vial for its activation | Store at 4–8°C |
| Buffer D<br>(Detachment Concentrate) | | Detachment of target from pluriBeads | Store at –20°C |
| M-pluriStrainer® / 60 µm<br>(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 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®

Gloves

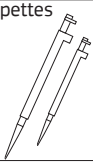
Mixer*



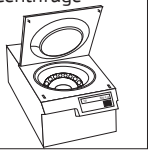
*No orbital shakers or laboratory rockers!

Tubes

Pipettes



Centrifuge

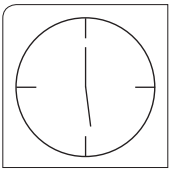


# 0. Coupling own Antibodies to Universal pluriBeads®*

## 0.1 Coupling with own Antibody

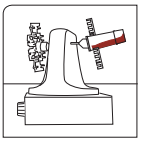
- Use Universal pluriBeads®, your own antibody and PBS solution at room temperature.
- Mix reagents in a 1.5 or 2 ml reaction tube.

| | |
|---|---|
| M-pluriBead® suspension | 1,000 µl (1x10$^6$ beads for 3x10$^7$ targets)** |
| - Supernatant | Pellet the beads (centrifuge 2 min at 5,000 x g without brake, if possible) and remove ~250 µl supernatant. |
| + Amount antibody | Minimum 20 µg antibody |
| + Volume PBS | Fill up with PBS to 1,000 µl total volume |
| = Total volume | = 1,000 µl |



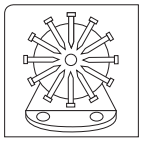
3-4 h

- Incubate antibody-pluriBead-solution 3–4 h at room temperature. pluriBeads® must remain in suspension during incubation.



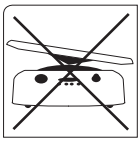
pluriPlix®/overhead >= 20 rpm

Thermoshaker/Rocker ~750 rpm



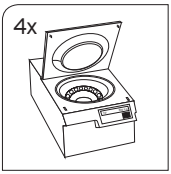
Rotator mixer

Horizontal roller mixer



Orbital shaker

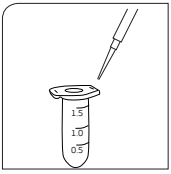
## 0.2 Washing




- Centrifuge the antibody-pluriBead®-solution and remove supernatant.

1. Centrifuge for 2 min at 5,000 x g without brake (if possible).
2. Carefully remove ~700 µl supernatant.
3. Add 1.20 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 1.20 ml supernatant in step 2.
6. Centrifuge for 2 min at 5,000 x g without brake (if possible).
7. Carefully remove 1.20 ml supernatant.

## 0.3 Usage / Storage



- **Immediate use:** Add 700 µl PBS solution (pH 7.4) onto the pellet.
- **Long-term storage** (max. 6 months at 4°C): Add 700 µl PBS solution with 0.05% sodium azide and 0.1% BSA onto the pellet and resuspend.

Both ways, you obtain ~1 ml suspension Universal pluriBeads coupled with your own antibody (~1x10$^6$ beads).

*Universal pluriBeads® can be coupled with any own antibody and subsequently can be applied according to the standard pluriBead® protocol (see pp. 7–10).
**Efficiencies of labeled pluriBeads® may vary depending on the antibody used.

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

## Buffers

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

## Consumables

| Product | Order No. | Size |
|---|---|---|
| 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 |

Distributed by:
ImTec DIAGNOSTICS NV
Contact: info@imtec.be | +32(0)3-2251139