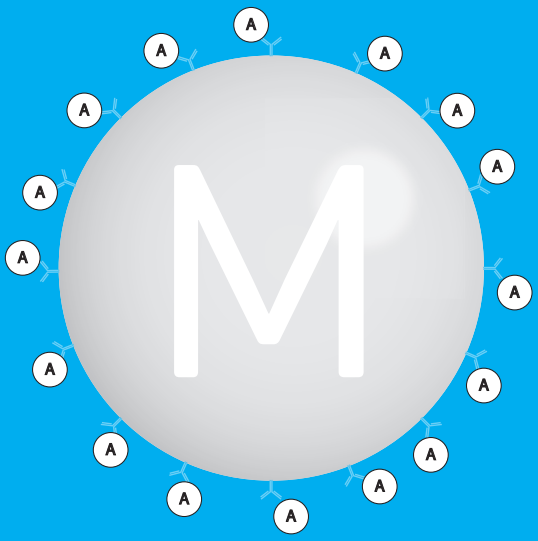
M-pluriBead®

Cell Separation Protocol

MANUAL



A - Target cells

pluriSelect

support@pluriselect.com | www.pluriselect.com

New Detachment Protocol

Contents

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

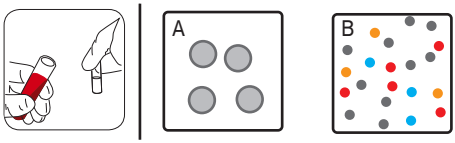
pluriBead® should be used for research use only.

pluriBead® Principle

Raw Material

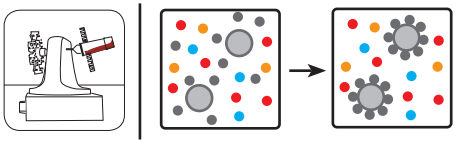
A – pluriBeads® with target-specific mAb coating.

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



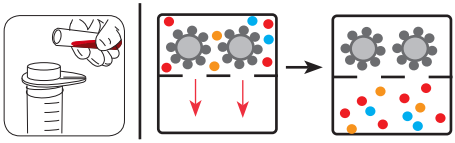
1. Incubation

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



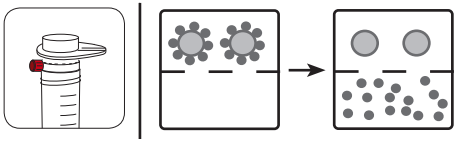
2. Washing

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.

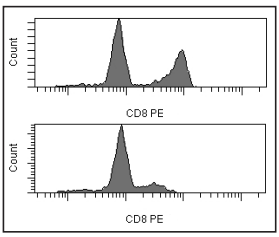


3. Detachment

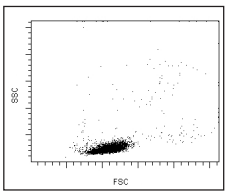
Target cells are detached from the beads with a detachment buffer directly on the strainer. They are then washed into a fresh tube while the depleted beads remain on the strainer.



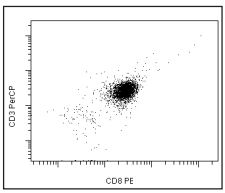
Typical pluriBead® Cell Separation Profile: Example CD8



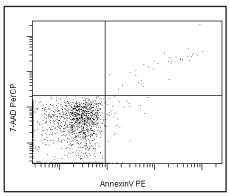
Histogram of whole blood before (top) and after (below) depletion



FSC/SSC analysis of isolated population



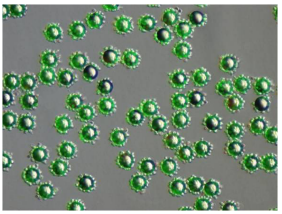
Fluorescent labeled isolated population



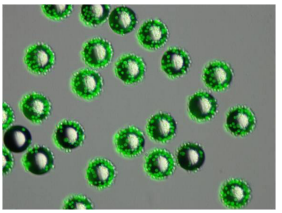
Apoptosis staining of isolated population

pluriBead® in Detail

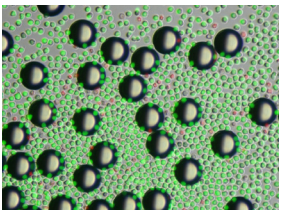
pluriBead® Particles - Phase Contrast



S-pluriBead®



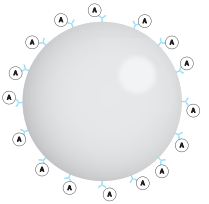
M-pluriBead®



Cells after detachment

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

pluriBead® Size

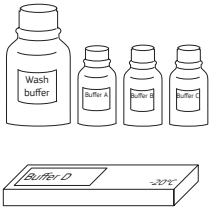
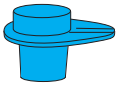
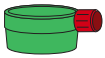
Specification	S-pluriBead®	M-pluriBead®
Illustration	 A - target cell	 A - target cell
pluriBead® Size	32 µm	65 µm
Maximum isolated cells per separation	1x 10^7	5x 10^7
Maximum bead suspension per pluriStrainer	400 µl	1,000 µl
Recommended application	medium number (≤2x10^6) of targets, rare cells, and 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 (M-pluriBeads - blue cap)		Catcher particles labeled with specific antibodies	Store at 4-8°C

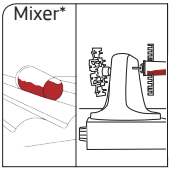
pluriBead® Reagent Kit

Kit Components	Picture	Description	Storage Conditions
Wash Buffer (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
M-pluriStrainer® / 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 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

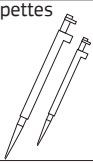
Mixer*



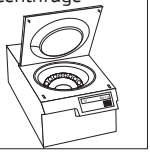
*No orbital shakers or laboratory rockers!

Tubes

Pipettes



Centrifuge

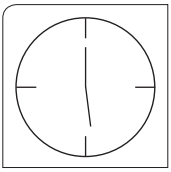


0. Coupling own Antibodies to Universal pluriBeads®*

0.1 Coupling with own Antibody

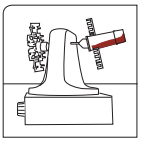
- Use Universal pluriBeads®, your own antibody and PBS solution at room temperature.
- Mix reagents in a 1.5 or 2 ml reaction tube.

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



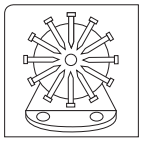
3-4 h

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



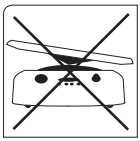
pluriPlix®/overhead >= 20 rpm

Thermoshaker/Rocker ~750 rpm



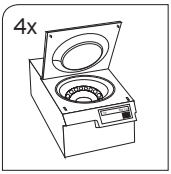
Rotator mixer

Horizontal roller mixer



Orbital shaker

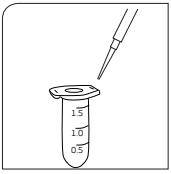
0.2 Washing



- 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



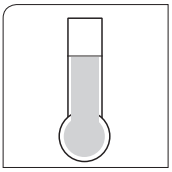
- **Immediate use:** Add 700 µl PBS solution (pH 7.4) onto the pellet.
- **Long-term storage** (max. 6 months at 4°C): Add 700 µl PBS solution with 0.05% sodium azide and 0.1% BSA onto the pellet and resuspend.

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.

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 (D): 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

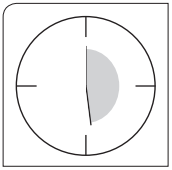
Whole Blood	Tissue/PBMC	Buffy Coat
• ***Add 50 µl*** of provided ***Buffer A*** per 1 ml sample. • **CD14:** Before separating CD14+ cells from a sample, ***remove sCD14!*** (see p. 11) • ***Mouse*** whole blood: ***Pre-filter*** sample to remove aggregates.	• Prepare a ***single cell suspension***. *Take up the cell pellet in 500 µl Buffer B and 500 µl Wash Buffer.* *Adjust the concentration of cells at max. 5x10⁶* **targets** *per 1 ml buffer.* • ***Pre-filter*** sample with S-pluriStrainer® to remove aggregates.	• ***Add 50 µl*** of provided ***Buffer A*** per 1 ml sample. • ***Pre-filter*** buffy coat. *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, prepare first PBMC or use another buffy coat!* • ***Alternatively use Buffy Coat Add-On*** *(Order.No.: 01-00600-10)*

1.3 Binding of Targets

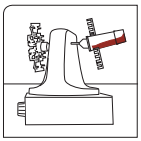
- Thoroughly ***resuspend pluriBead®*** suspension by vortexing the tube.
- Add pluriBead® suspension and sample into a sterile mixing tube:

Whole blood:	*50 µl M-pluriBead® suspension per 1 ml*
Other samples:	*50 µl M-pluriBead® suspension per 1.5x10⁶ targets*
Alternatively:	*50 µl M-pluriBead® suspension per 1x10⁶ total cells*

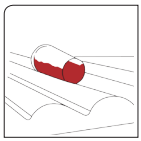
Recommended tube sizes:
Sample + pluriBeads® 0.2–2 ml: 2 ml tube (***round bottom***)
Sample + pluriBeads® 2–6 ml: 15 ml tube or use the blood collection tube
Sample + pluriBeads® 6–50 ml: 50 ml tube



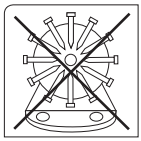
- ***Incubate mixture*** up to 30 min. pluriBeads® must remain in suspension.



pluriPlix®
(by pluriSelect)
10–15 rpm/7.5° angle

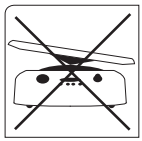


Horizontal roller mixer
(with tilting function)
10–15 rpm



Rotator mixer

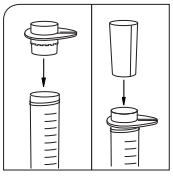
Laboratory rocker



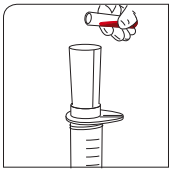
Orbital shaker

2. Washing

Small Suspension M-pluriBead® suspension <=1 ml



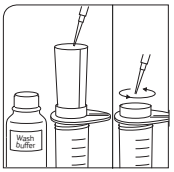
· ***Attach a M-pluriStrainer®*** to a sterile ***50 ml*** centrifuge ***tube***.
The bigger opening of the strainer must be on the top.
· ***Optional for sample more than 4ml: Place*** the supplied ***funnel*** on top of the strainer .
Maximum application volume of liquid on the strainer increases to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



· ***Pour sample*** (more than 4 ml) onto the pluriStrainer.

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

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



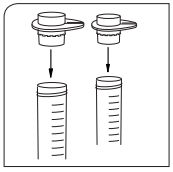
· Recommendation: Use a 5 ml pipette (no serological pipette).
· ***Wash*** off the bead-sample traces from the ***pluriStrainer*** / ***funnel*** in 2 ml steps*
and discard the funnel.
· ***Wash the strainer*** sufficiently in 2 ml steps 1x Wash Buffer.
· Wash in a ***circular motion***, avoid washing the middle of the strainer only.

We recommend washing with at least 20 ml in total*.

Watch out the tube beneath the strainer 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.

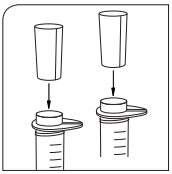
Large Suspension M-pluriBead® suspension >1 ml



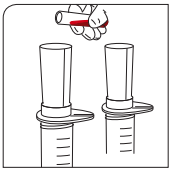
· According to the applied amount of pluriBead® suspension, use ***several M-pluriStrainers®*** (see table below).
Attach each strainer to a sterile 50 ml centrifuge tube. The bigger opening must be on top.

Total volume of M-pluriBead® suspension in your sample	1 ml	2 ml	3 ml
No. of strainers and tubes to be applied	1x	2x	3x

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



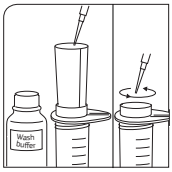
- ***Place*** the supplied ***funnels*** on top of the strainers.
  Maximum application volume of liquid on the strainer increases to 15 ml.
- Add 1 ml Wash Buffer each to equilibrate the strainers and to support separation.



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

Unbound cells run through the strainer into the centrifuge tube, the bead-bound 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.

We recommend washing with at least 20 ml in total*.

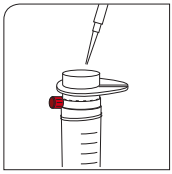
Watch out that the tubes beneath the strainers contain no more than 45 ml of sample/Wash Buffer! Use several tubes if necessary

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

After washing pluriStrainer®, *erythrocytes or other unwanted material* might still adhere to the inner surface of the pluriStrainer. 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 pluriStrainer.

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.

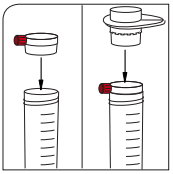
- Attach the connector tightly to a new 50 ml centrifuge tube.
- Close the Luer-Lock and attach the strainer with the isolated target cells.
  Caution: Do not tilt the strainer!
- Add lysis buffer according to the manufacturers direction of use.

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

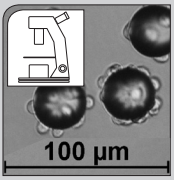
3. Detachment

3. Detachment

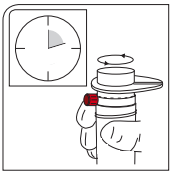
(Target cells will be released from the pluriBeads into the tube.)



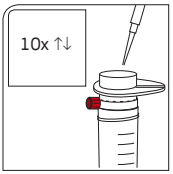
- ***Attach the connector*** straight and tight onto a new 50 ml tube.
- ***Attach and close the Luer-Lock.***
- ***Attach the strainer*** with the isolated target cells to the connector ring. Caution: Fit even and do not tilt the pluriStrainer!
- ***Add 1 ml Wash Buffer*** along the wall of the strainer.



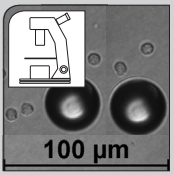
Optional check 1 – *Check if the target cells are bound to the beads.*
- *Resuspend the beads by gently moving the strainer in a circular way.*
- *Retain a small amount of sample (10 µl) and place it on a microscope slide or in a microwell plate.*
- *Check if the target cells are bound to the beads.*



- ***Add 1 ml activated Buffer D*** (200 µl Buffer C into 1 vial Buffer D) 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.

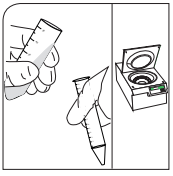


- After incubation, add ***1 ml Wash Buffer***.
- Separate the cells from the beads by ***pipetting up and down*** the sample on the strainer with a 1 ml pipette (10x). Avoid air bubbles and do not touch the mesh with the pipette.



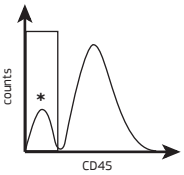
Optional check 2 – *Check if the cells are released from the beads.*
- *Retain a small amount of sample (10 µl) and place it on a microscope slide or in a microwell plate.*
- *Check under the microscope if the cells are released from the beads.*
- *If cells are still bound to the pluriBeads®, extend 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 50 ml centrifuge tube, pluriBeads® remain on the pluriStrainer®.
- ***Wash the strainer 10 times*** with 1 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.

Common Error Sources

Target Yield	100 µl M-pluriBead® suspension can bind up to $5x10^6$ 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. 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 aggregates 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 (Stablization 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
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

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

Manual_M_pluriBead_EN_V5-7