Preparing Cells for EasySep™

Common Human Peripheral Blood Samples and How to Use Them with EasySep™

Presenter
LU LU
SCIENTIFIC SUPPORT SPECIALIST, IMMUNOLOGY

STEMCELL™ TECHNOLOGIES

Learning Objectives

In this session, you will learn:

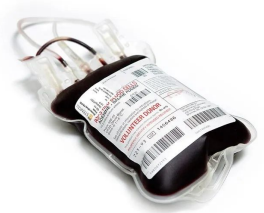
- Which common human peripheral blood samples are compatible with EasySep™
- How to use human blood samples with EasySep™
- How to process leukopaks (LPs) for EasySep™
- How to process Leukocyte Reduction System Cones (LRSCs) for EasySep™

Common Human Peripheral Blood Samples Compatible with EasySep™

Common Human Peripheral Blood Samples Compatible with EasySep™

Unprocessed

Whole Peripheral Blood

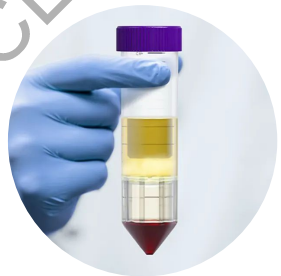


Pre-Processed

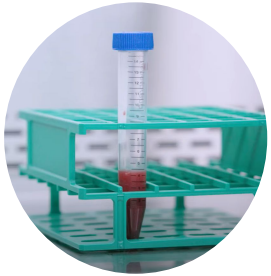
Leukopak (LP)

Leukocyte Reduction System Cone (LRSC)

Peripheral Blood Mononuclear Cells (PBMCs)



Buffy Coat

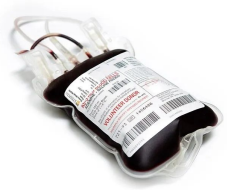


*Certain products are only available in select territories. Please contact your local Sales representative or the Product & Scientific Support team at techsupport@stemcell.com for further information.

STEMCELL™ TECHNOLOGIES

Whole Blood and Apheresis Blood Samples

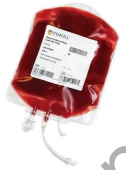
Whole Peripheral Blood



storage at room temperature

Common apheresis blood products

- **Leukopak (LP)**



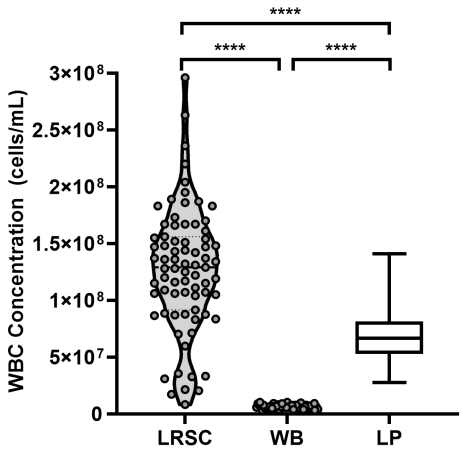
Fresh
storage at 2 - 8°C

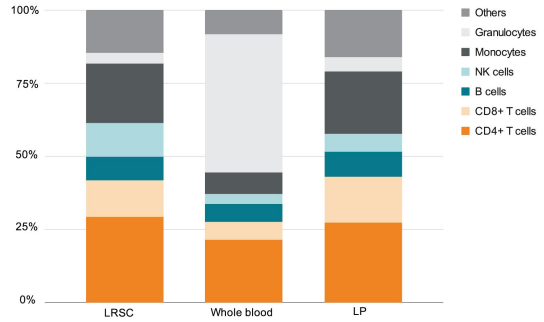
Frozen
storage at -135°C or cooler

- **Leukocyte Reduction System Cone (LRSC)**

storage at 2 - 8°C

Apheresis blood samples are collected using a process called "leukapheresis". Compared to whole blood, they are rich in leukocytes and low in RBCs and granulocytes.



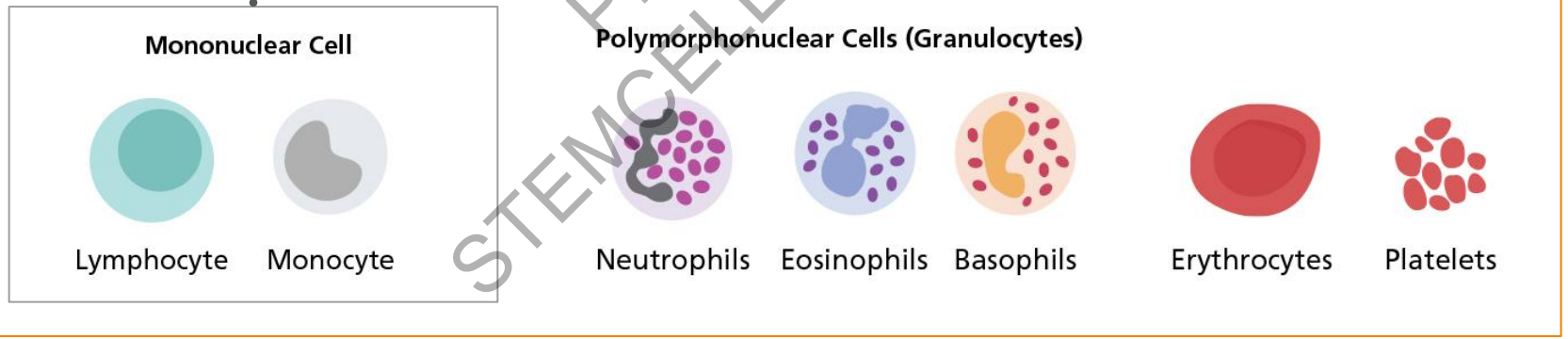


STEMCELL™ TECHNOLOGIES

PBMCs and Buffy Coat

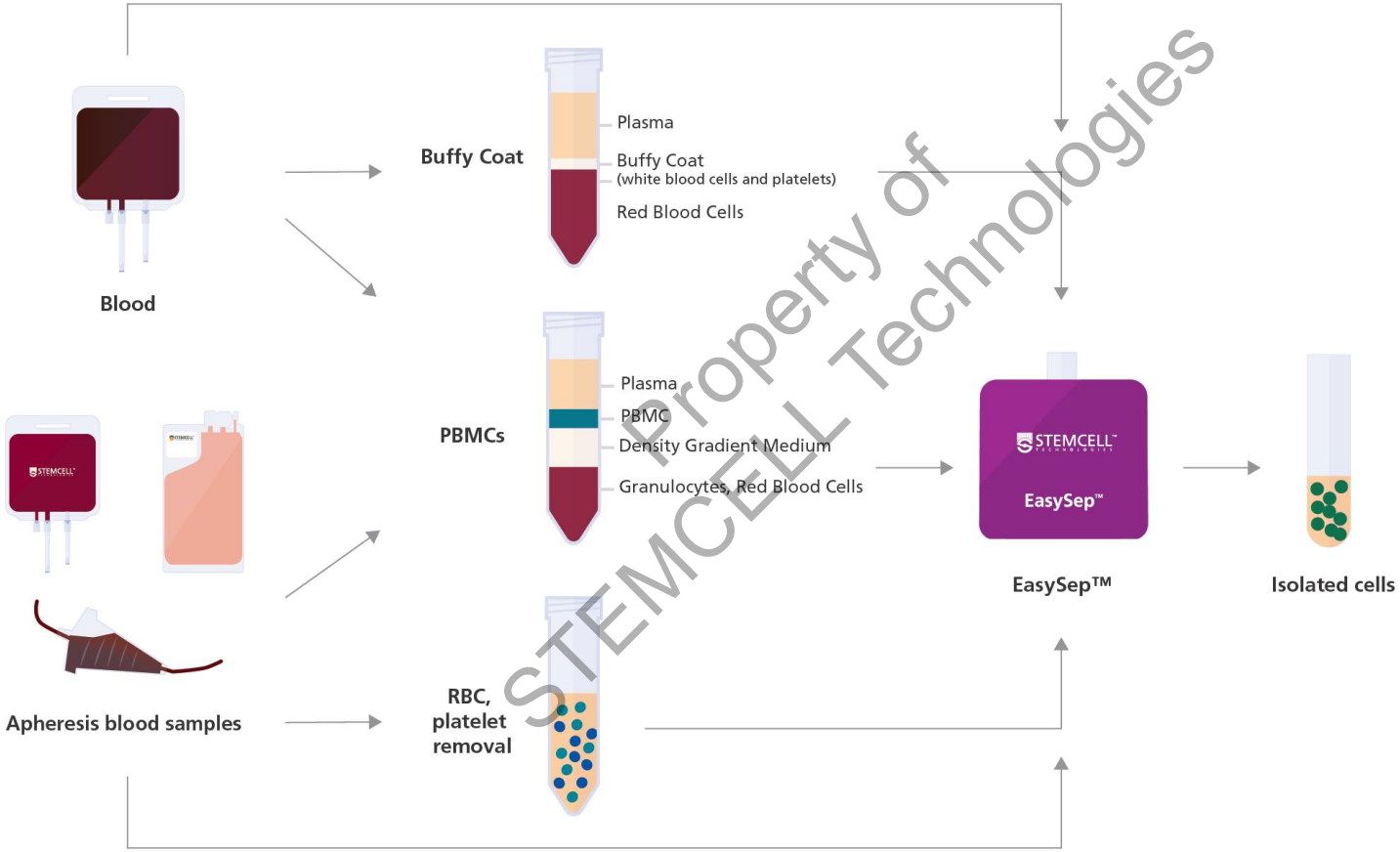
PBMCs
- Lymphocytes (T cells, B cells, and NK cells)
- Monocytes, Dendritic cells

Buffy Coat
- PBMCs
- Granulocytes
- Erythrocytes (RBCs)
- Platelets



How to Use Human Peripheral Blood Samples with EasySep™

How to Use Human Blood Samples with EasySep™



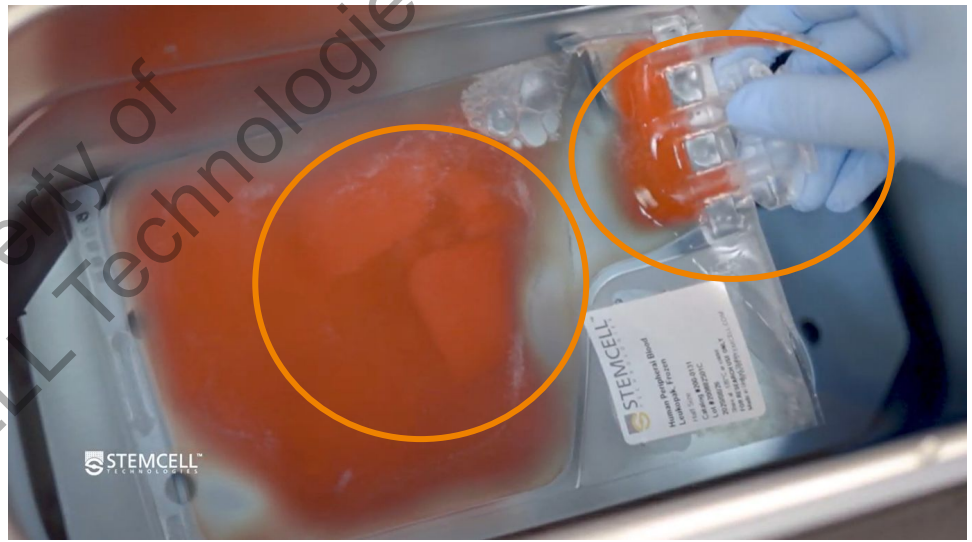
How to Process Leukopaks (LPs)

Thawing a Frozen LP

Note: Inspect the bag and lines.

1. Prepare thawing medium (HBSS Modified + 10% FBS + 100 µg/mL DNase I) and warm up.
    - Thawing medium must be prepared fresh before use.
2. Remove LP from liquid nitrogen storage and immediately place in a 37°C water bath.
    - LP should be submerged in the water, but keep the ports and tubing above the waterline.
    - LP should not be agitated during thaw.
3. Remove LP when it is mostly (**not completely**) thawed.

Step 2. Thawing LP in a 37°C water bath



STEMCELL™ TECHNOLOGIES

Thawing a Frozen LP

Note: Inspect the bag and lines.

1. Prepare thawing medium (HBSS Modified + 10% FBS + 100 µg/mL DNase I) and warm up.
   - Thawing Medium must be prepared fresh before use.
2. Remove LP from liquid nitrogen storage and thaw with the ThawSTAR® CB Automated Thawing System.

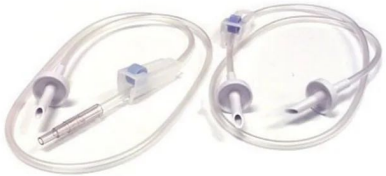
Step 2. ThawSTAR® CB Automated Thawing System

Recovering Content from an LP

Surface disinfect the LP. Inside a Biosafety Cabinet (BSC), transfer leukopak contents to a sterile container.

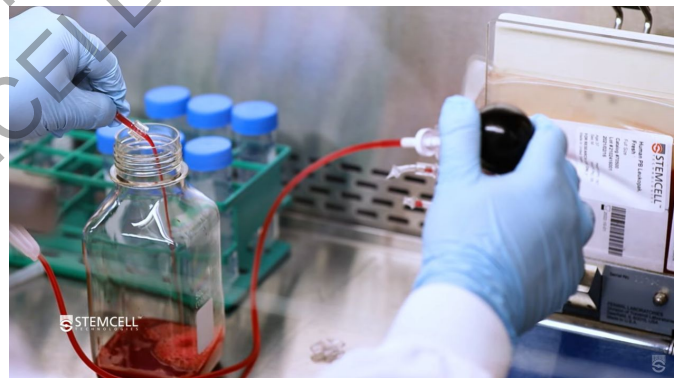
- Blood transfer kit should be sterile and possess a spike connector/coupler.
- Use the outlet port with a universal spike connector.
- Apply pressure to the LP through an extractor stand, press the bag, or use gravity to drain the bag.
- Sterile container should be ≥ 2x LP content volume for fresh LP and ≥ 5x volume for frozen LP.
- Optional: Cut the bag and rinse it with buffer (PBS+2%FBS or Thawing Medium) to maximize the recovery. Use extra care with this step to avoid introducing contamination.

Transfer kit and outlet port



Andwin Scientific Fenwal Plasma Transfer Set

Apply pressure to the LP through an extractor stand



STEMCELL™ TECHNOLOGIES

Washing the Content from an LP

For Frozen LP

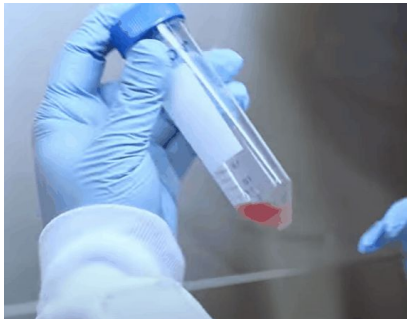
1. Slowly add three volumes of thawing medium dropwise while swirling the bottle.
2. Distribute cell suspension into 50 mL conical tubes. Pellet the cells (300 x *g*, 10 minutes, RT, break ON). Aspirate the supernatant and loosen the pellet by gently flicking the tube.
    - Optional: To prevent cell aggregation, add 100 µg DNase I per mL of cell suspension and incubate for 15 minutes at room temperature.
3. Wash the cells again with the thawing medium and centrifuge at 300 x *g*. Aspirate the supernatant and loosen the pellet.
    - If desired, pool all the cells from the different tubes. Make sure to rinse the tubes to maximize recovery.

For Fresh LP

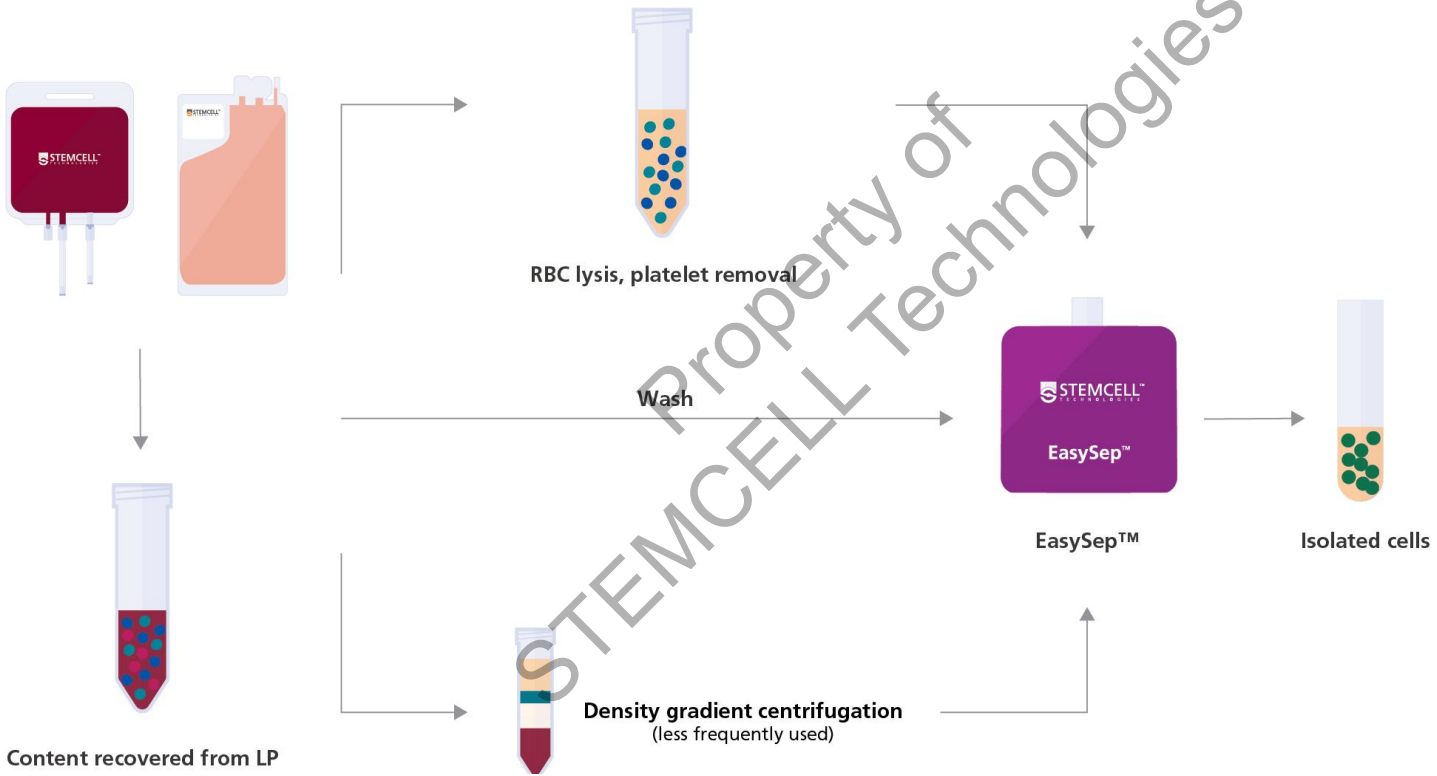
1. Add an equivalent volume of PBS + 2% FBS.
2. Distribute cell suspension into 50 mL conical tubes. Centrifuge the cells (300 x *g*, 10 minutes, RT, break ON) and resuspend with recommended medium.

Step 1: Add thawing medium dropwise while swirling the bottle

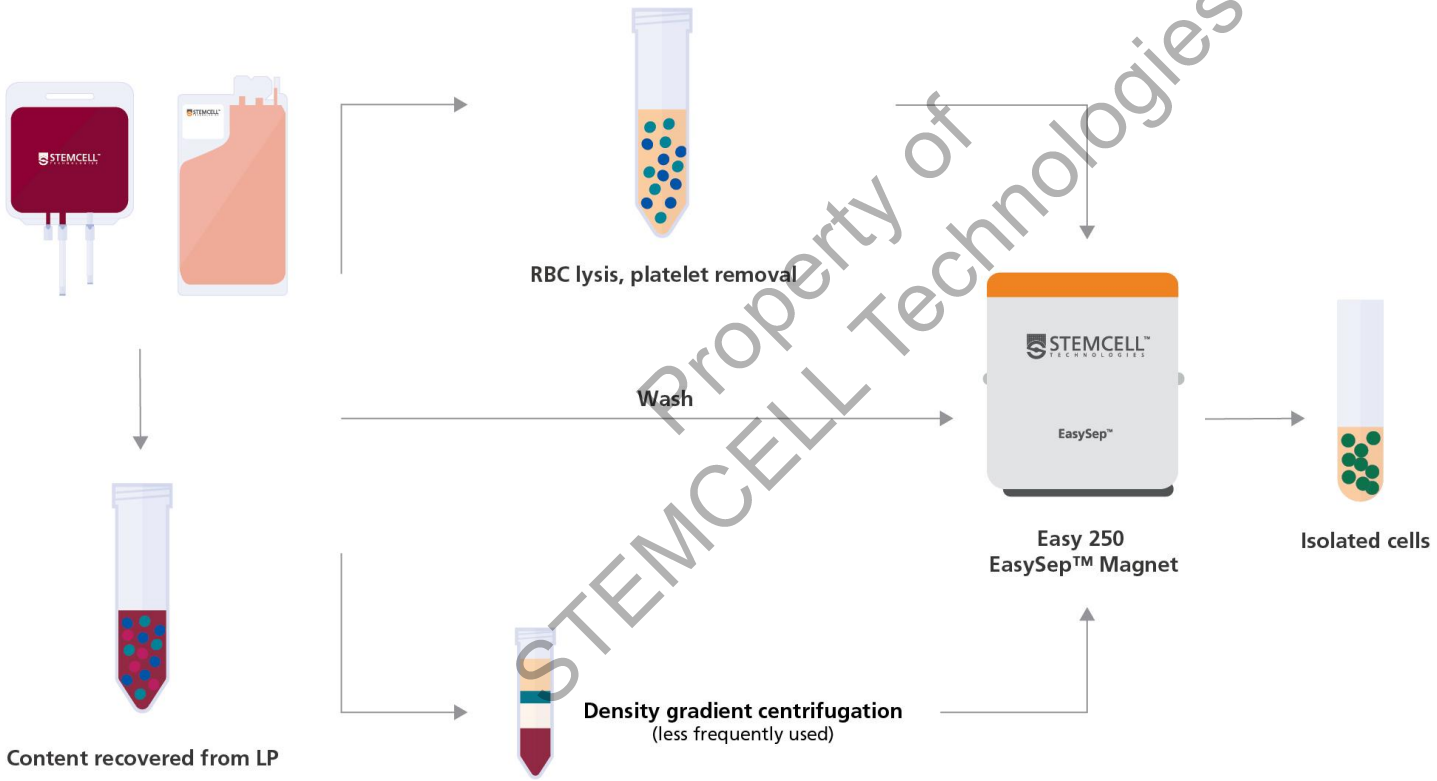
Step 2: Loosen the pellet by gently flicking the tube



Processing Content from an LP for EasySep™ Cell Isolation



Processing Content from an LP for EasySep™ Cell Isolation



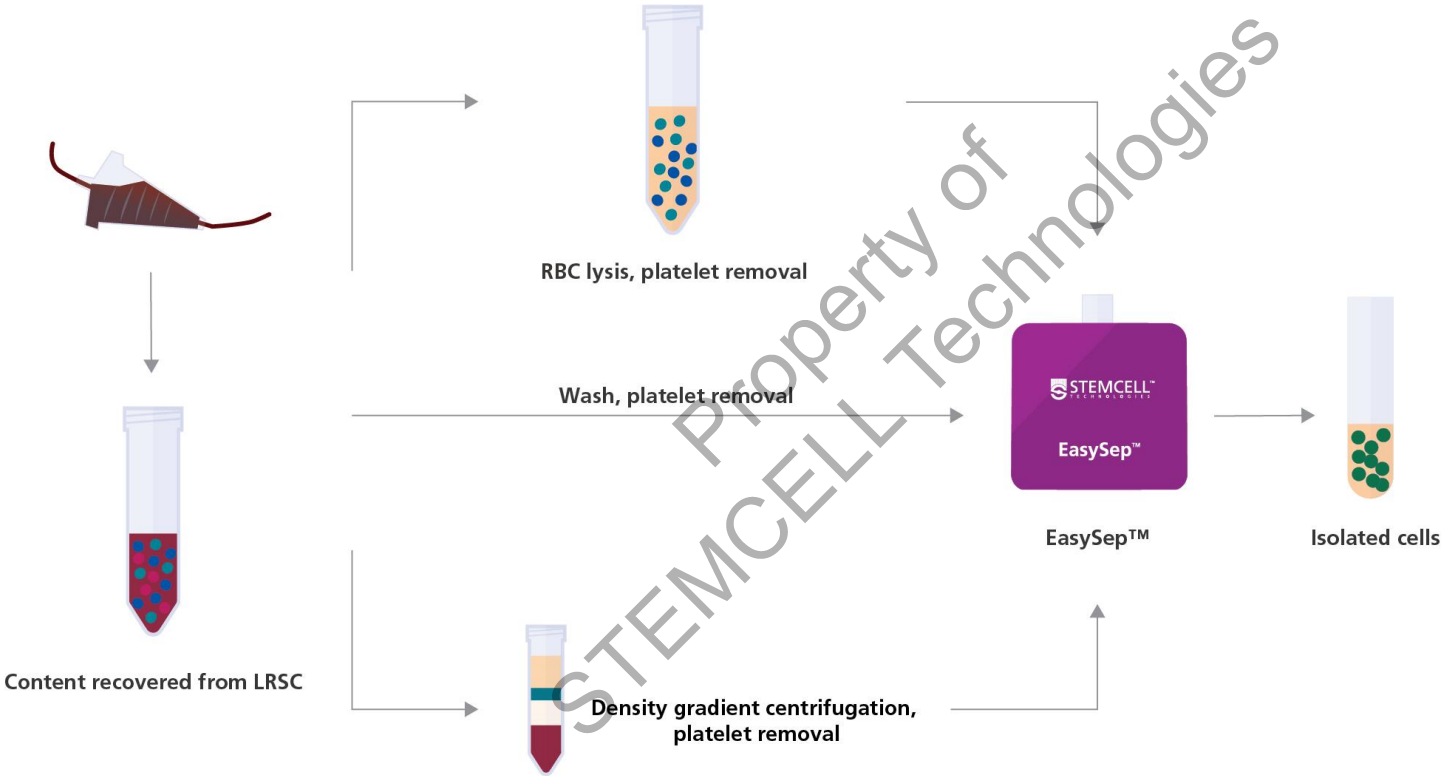
STEMCELL™ TECHNOLOGIES

How to Process Leukocyte Reduction System Cones (LRSCs)

Recovering Content from an LRSC

1. Cut the tubing on the wide side of the LRS cone, place the cone wide side down over a 50 mL conical tube.
   - Use sterile scissors and hold the cone over the collection tube when cutting the tubing.
2. Cut the upper tubing (leave ~1 cm of tubing) to let the contents drip into the tube.
3. Place a 200 µL pipette tip into the top tubing. Then, attach a blunt-end 16-gauge needle on a syringe and insert into the upper tubing through the pipette tip.
4. Flush air into the cone to facilitate sample collection.
5. Wash the cone with ~10 mL buffer (PBS containing 2% FBS).
   - Gently dispense the buffer at a rate of ~2 mL/sec.
   - Move the syringe in a circular motion to wash the sides of the cone.
6. Flush air into the cone again to facilitate sample collection.
7. Repeat steps 5-6 two more times to maximize the recovery. The sides of the cone should look clear.

Processing Content from LRSC for EasySep™ Cell Isolation

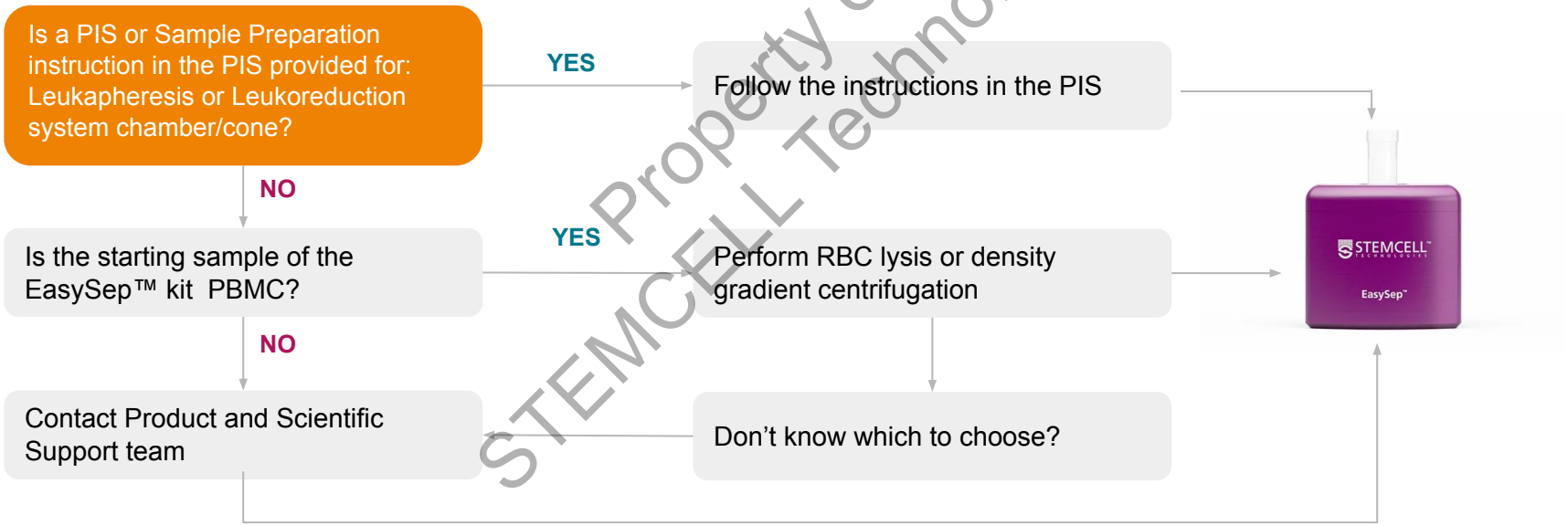


STEMCELL™ TECHNOLOGIES

Processing Content from LPs/LRSCs for EasySep™ Cell Isolation

Knowing which protocol to follow:

Always consult the Product Information Sheet (PIS) of the product;
Consider the purity requirement and compatibility with the processing methods for downstream applications.



STEMCELL™ TECHNOLOGIES

Summary

- Whole peripheral blood can be used directly with some EasySep™ products or processed to remove certain components prior to EasySep™ separation.
- Apheresis blood samples are generated during the process of leukapheresis. They are rich in leukocytes and low in RBCs and granulocytes. Common apheresis blood products compatible with EasySep™ include leukopaks (LPs) and Leukocyte Reduction System Cones (LRSCs).
- PBMCs and buffy coat have different compositions, each can be used with some EasySep™ products.
- Content recovered from fresh or thawed frozen LPs can be used with EasySep™ after wash, RBC lysis, or density gradient separation. DNase I is added to the thawing medium for frozen LPs to reduce cell aggregation. Additional DNase I treatment and filtering can be performed after the initial wash step to further reduce cell aggregation.
- Content recovered from LRSCs can be used with EasySep™ after washing, density gradient separation, or RBC lysis. RBC and platelet removal is recommended prior to most EasySep™ products.

Questions?

Scientists Helping Scientists™ | TECHSUPPORT@STEMCELL.COM

Thank you!

Scientists Helping Scientists™ | WWW.STEMCELL.COM

Please complete the quiz before moving onto Module 2.