EasySep™ Technology

Key Steps

Presenter
LU LU
SCIENTIFIC SUPPORT SPECIALIST, IMMUNOLOGY

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Learning Objectives

In this session, you will learn:

How to execute the key steps when performing cell separation with EasySep™

- Before cell separation with EasySep™:
  - Storing the kit components
  - Preparing the starting sample
  - Selecting a magnet
- During cell separation with EasySep™:
  - Adding reagents and buffer
  - Removing the supernatant after magnetic separation
  - Resuspending the particle-bound cells

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Before Cell Separation with EasySep™

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Storing EasySep™ kit components

- EasySep™ selection cocktails and magnetic particles should be stored at 2 - 8°C.
  - Use a cold room or fridge to store products at 2 - 8°C. Ensure the reagents do not freeze.
  - Some EasySep™ kit components may need to be stored at other temperatures; please check the storage instructions of the products upon receipt.
- Always check the expiry date of the reagents before starting your experiment.
- Compromised, frozen, or expired products will affect the performance of separation.

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Starting Sample: a Single-Cell Suspension with Suggested Concentration, in a Recommended Buffer

- A **single-cell** suspension enables maximum labeling of the target cells and optimal separation in the magnet.
- The **concentration of cells** also affects the labeling efficiency and separation efficacy.
- Buffer provides the environment for labeling and separation; use a **recommended buffer** for optimal separation.

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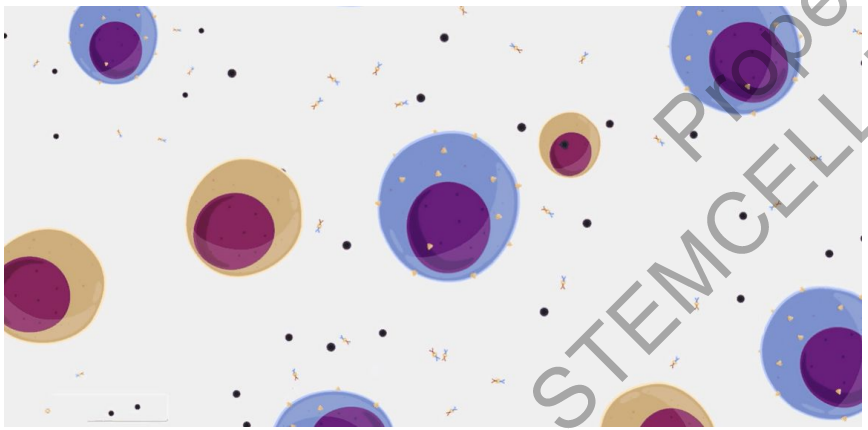
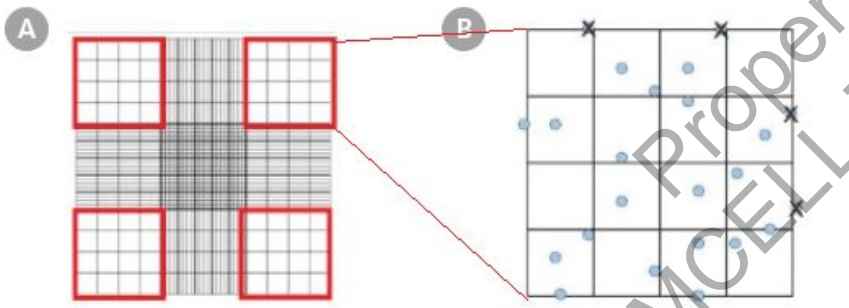
Labeling of target cells



Magnetic separation of cells

Determining Cell Concentration and Total Cell Count

- Dyes for cell counting:
  - Trypan Blue can be used to determine the number of cells and viable cells.
  - 3% Acetic Acid with Methylene Blue lyses cell membranes and stains the remaining nuclei blue. It is recommended when determining total nucleated cell count.
- We recommend counting stained cells manually with a hemocytometer.



Cell Concentration (Number of cells/mL)
= average cell count per square x dilution factor x 10^4

Total Cell Count
= Cell Concentration (number of cells/mL) x volume of sample (mL)

- Pay attention to the algorithm and settings if you are using an automatic cell counter.

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Adjusting Cell Concentration

1. Check the cell concentration suggested in the Product Information Sheet (PIS).
2. Calculate the volume of the starting sample by:

$$\textbf{Total volume of starting sample (mL)} = \frac{\text{Total Cell Count}}{\text{cell concentration suggested in PIS (cells/mL)}}$$

Volume of buffer to be added (mL) = Total volume of starting sample (mL) - volume of counted sample (mL)

- Use the recommended buffer
- Save a small portion of the starting sample for target cell frequency analysis

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Cell Separation with EasySep™

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Selecting a Magnet

Based on the starting sample volume, select an EasySep™ magnet compatible with your EasySep™ kit and sample volume.

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- Use a tube/flask/plate recommended in the protocol with the corresponding magnet.
- If the adjusted starting sample volume > the magnet's recommended volume:
  - Check if the kit is compatible with a magnet suited to process larger sample volumes; or
  - Split the starting sample.
- If the adjusted starting sample volume < the magnet's recommended volume:
  - Check if the kit is compatible with a magnet suited to process smaller sample volumes; or
  - Top up the starting sample to the minimum volume of the magnet.

Adding Reagents and Buffer

- Check all the reagents; ensure they are not expired or compromised. Pay special attention to the particles; invert the vial to make sure there are no large aggregates.
- Vortex the magnetic particles for 30 seconds right before use.
- Follow the suggested volume when adding reagents or topping up with the recommended buffer.
- When adding reagents, the end of the tip should go into the solution to make sure everything is added. Mix the solution with at least half of the total sample volume after adding the reagent.
- Time and temperature of incubations are critical;
  follow the instructions in the Product Information Sheet.

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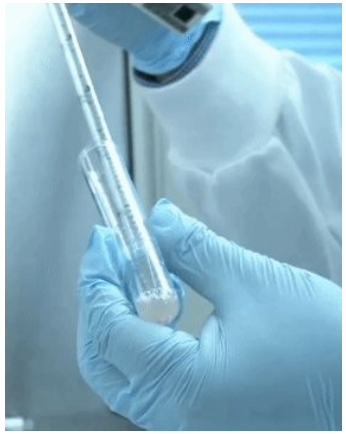
Vortex magnetic particles

Add reagents

Mix



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Removing the Supernatant During Separation

Pour off

Pipette off

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EasySep™ Magnet and "The Big Easy" EasySep™ Magnet

Pour off

- Keep the tube inserted to the bottom of the magnet
- Ensure the collection tube and separation tube do not touch
- Pour in one continuous motion
- Wait for 2 - 3 seconds in the inverted position
- Do not shake off the last drop

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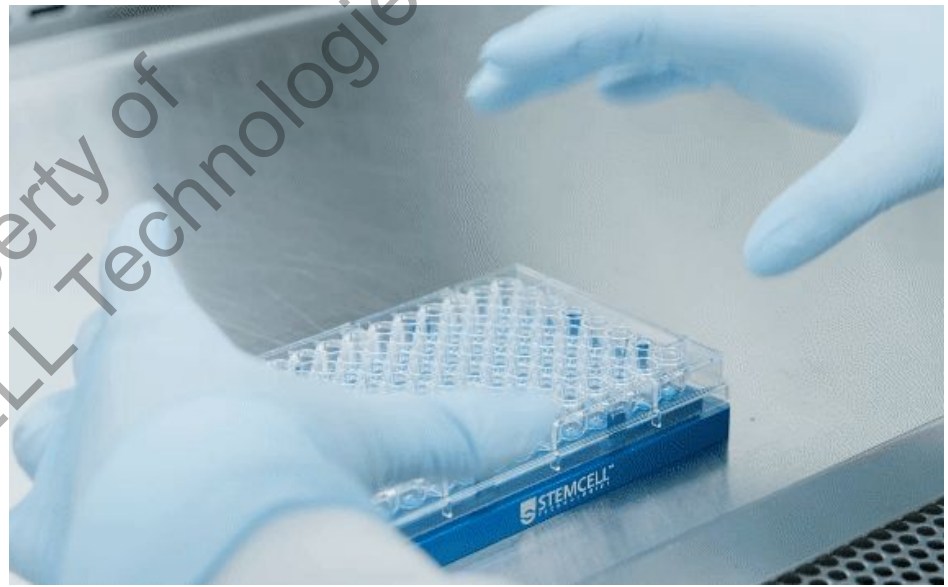
EasyPlate™ EasySep™ Magnet

Pipette off

- Ensure the 96-well plate sits securely on the EasyPlate™ magnet
- Carefully tilt the plate and magnet together at a 45° angle
- Use a single or multichannel pipette to carefully collect the supernatant out of each well
- Do not touch the bottom of the well with the pipette tip



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Easy 50 EasySep™ Magnet

Pipette off

- Make sure the tube is properly seated in the magnet and do not move
- Use a 25 mL serological pipette to collect the supernatant
- Keep the tip of the pipette just below the liquid surface while aspirating
- Do not touch the particle-bound cells on the magnet side of the tube

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EasyEights™ EasySep™ Magnet

Pipette off

- Make sure the tube is properly seated in the magnet and do not move
- Use a 2 or 10 mL serological pipette to collect the supernatant
- Keep the tip of the pipette just below the liquid surface while aspirating
- Do not touch the particle-bound cells on the magnet side of the tube

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Easy 250 EasySep™ Magnet

Pipette off

- Place a finger on the flask shoulder to stabilize it
- Use a 25 mL serological pipette to collect the bulk supernatant
- Keep the tip of the pipette just below the liquid surface while aspirating
- Use a smaller serological pipette to draw up the residual volume
- Do not touch the particle-bound cells on the magnet side of the flask

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Resuspending Magnetically Labeled Cells

1. Agitate the tube to loosen the cells

2. Add buffer along tube walls to wash the cells; repeat the wash a few times

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Summary

- It is important to follow the storage instructions of the EasySep™ kit and use the reagents before expiry to ensure the separation performs as expected.
- The starting sample should be a true single-cell suspension, adjusted to the concentration suggested in the Product Information sheet, in a recommended buffer.
- You should choose a magnet based on the compatibility with the EasySep™ kit and the starting sample volume.
- Follow the instructions in the Product Information Sheet for the volume of reagents, incubation time, and temperature. Check and vortex the magnetic particles before use.
- Gently but sufficiently mix the sample with the reagents after each addition to ensure optimal labeling.
- Remove the supernatant from the tube with the proper collection method.
- When resuspending the particle-bound cells, agitate the tube and wash it thoroughly with recommended buffer.

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Questions?

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Thank you!

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Please complete the quiz before moving onto Module 5.