



## EasySep™ Mouse CD4 Positive Selection Kit II

Positive Selection

Catalog #18952

For labeling up to 2 x 10<sup>9</sup> cells



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## Description

Isolate highly purified CD4<sup>+</sup> cells from mouse splenocytes or other single-cell suspensions by positive selection.

- Fast and easy-to-use
- Up to 99% purity
- No columns required
- Isolated cells are not fluorescently labeled

This kit targets CD4<sup>+</sup> cells for positive selection with antibodies recognizing the CD4 surface marker. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, culture and cell-based experiments.

## Component Descriptions

| COMPONENT NAME                                           | COMPONENT # | QUANTITY   | STORAGE                          | SHELF LIFE                               | FORMAT                                                      |
|----------------------------------------------------------|-------------|------------|----------------------------------|------------------------------------------|-------------------------------------------------------------|
| EasySep™ Mouse CD4 Positive Selection Kit II Component A | 18952CA     | 1 x 0.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS and 0.1% BSA. |
| EasySep™ Mouse CD4 Positive Selection Kit II Component B | 18952CB     | 1 x 0.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS and 0.1% BSA. |
| EasySep™ Dextran RapidSpheres™ 50100                     | 50100       | 1 x 1 mL   | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water.                |
| Normal Rat Serum                                         | 13551       | 1 x 2 mL   | Store at -20°C.                  | Stable until expiry date (EXP) on label. | Mycoplasma-free normal rat serum.                           |
| RoboSep™ Empty Vial                                      | 27401       | 1          | Not applicable                   | Not applicable                           | Not applicable                                              |

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Additional Reagent Stability Information

| REAGENT NAME                                            | STORAGE                          | SHELF LIFE                                                                   |
|---------------------------------------------------------|----------------------------------|------------------------------------------------------------------------------|
| Selection Cocktail (combined Component A + Component B) | Store at 2 - 8°C. Do not freeze. | Stable for up to 4 weeks. Do not exceed shelf life of individual components. |
| Normal Rat Serum (in-use)                               | Store at 2 - 8°C.                | Stable for at least 2 months. Do not exceed expiry date (EXP) on label.      |

## Sample Preparation

Disrupt spleen in phosphate-buffered saline (PBS) or Hanks' Balanced Salt Solution containing 2% fetal bovine serum (FBS). Remove clumps and debris by passing the cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10<sup>8</sup> nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog 20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca<sup>++</sup> and Mg<sup>++</sup>.

## Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Mouse CD4 Positive Selection Kit II Instructions**

|      |                                                                                                                                                                                    | EASYSEP™ MAGNETS                                                                                                      |                                                                                                                                        |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| STEP | INSTRUCTIONS                                                                                                                                                                       |  <b>EasySep™</b><br>(Catalog #18000) | <b>“The Big Easy”</b><br>(Catalog #18001)           |
| 1    | Prepare sample at the indicated cell concentration within the volume range.                                                                                                        | 1 x 10e8 cells/mL<br>0.25 - 2 mL                                                                                      | 1 x 10e8 cells/mL<br>0.25 - 8 mL                                                                                                       |
|      | Add sample to required tube.                                                                                                                                                       | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Corning® Catalog #352058)                                    | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning® Catalog #352057)                                                   |
| 2    | Add Normal Rat Serum to sample.                                                                                                                                                    | 50 µL/mL of sample                                                                                                    | 50 µL/mL of sample                                                                                                                     |
| 3    | Prepare Selection Cocktail in a tube. For each 1 mL of sample make 50 µL of cocktail (25 µL of Component A + 25 µL of Component B).                                                | Mix equal volumes of Component A and Component B.<br>Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.        | Mix equal volumes of Component A and Component B.<br>Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.                         |
|      | Incubate.                                                                                                                                                                          | RT for 5 minutes                                                                                                      | RT for 5 minutes                                                                                                                       |
| 4    | Add Selection Cocktail to sample.                                                                                                                                                  | 50 µL/mL of sample                                                                                                    | 50 µL/mL of sample                                                                                                                     |
|      | Mix and incubate.                                                                                                                                                                  | RT for 5 minutes                                                                                                      | RT for 5 minutes                                                                                                                       |
| 5    | Vortex RapidSpheres™.                                                                                                                                                              | 30 seconds                                                                                                            | 30 seconds                                                                                                                             |
| 6    | Add RapidSpheres™ to sample.                                                                                                                                                       | 30 µL/mL of sample                                                                                                    | 50 µL/mL of sample                                                                                                                     |
|      | Mix and incubate.                                                                                                                                                                  | RT for 3 minutes                                                                                                      | RT for 3 minutes                                                                                                                       |
| 7    | Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                                                              | Top up to 2.5 mL                                                                                                      | <ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 2 mL</li> <li>• Top up to 10 mL for samples ≥ 2 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.                                                                                                                         | RT for 3 minutes                                                                                                      | RT for 3 minutes                                                                                                                       |
| 8    | Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant.* Remove the tube from the magnet; this tube contains the isolated cells. | Discard supernatant                                                                                                   | Discard supernatant                                                                                                                    |
| 9    | Repeat steps as indicated.                                                                                                                                                         | Steps 7 and 8, two more times<br>(total of 3 x 3-minute separations)                                                  | Steps 7 and 8, three more times<br>(total of 3 x 3-minute separations)                                                                 |
| 10   | Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.                                                                                            | Isolated cells are now ready for use                                                                                  | Isolated cells are now ready for use                                                                                                   |

RT - room temperature (15 - 25°C)

\* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

**Table 2. EasySep™ Mouse CD4 Positive Selection Kit II Instructions**

|      |                                                                                                                                              | EASYSEP™ MAGNETS                                                                                            |                                                                                                                                        |
|------|----------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| STEP | INSTRUCTIONS                                                                                                                                 | EasyEights™ (Catalog #18103)                                                                                |                                                                                                                                        |
|      |                                                                                                                                              | 5 mL tube                                                                                                   | 14 mL tube                                                                                                                             |
| 1    | Prepare sample at the indicated cell concentration within the volume range.                                                                  | 1 x 10e8 cells/mL<br>0.25 - 1 mL                                                                            | 1 x 10e8 cells/mL<br>0.5 - 8 mL                                                                                                        |
|      | Add sample to required tube.                                                                                                                 | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Corning® Catalog #352058)                          | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning® Catalog #352057)                                                   |
| 2    | Add Normal Rat Serum to sample.                                                                                                              | 50 µL/mL of sample                                                                                          | 50 µL/mL of sample                                                                                                                     |
| 3    | Prepare Selection Cocktail in a tube. For each 1 mL of sample make 50 µL of cocktail (25 µL of Component A + 25 µL of Component B).          | Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks. | Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.                            |
|      | Incubate.                                                                                                                                    | RT for 5 minutes                                                                                            | RT for 5 minutes                                                                                                                       |
| 4    | Add Selection Cocktail to sample.                                                                                                            | 50 µL/mL of sample                                                                                          | 50 µL/mL of sample                                                                                                                     |
|      | Mix and incubate.                                                                                                                            | RT for 5 minutes                                                                                            | RT for 5 minutes                                                                                                                       |
| 5    | Vortex RapidSpheres™.                                                                                                                        | 30 seconds                                                                                                  | 30 seconds                                                                                                                             |
| 6    | Add RapidSpheres™ to sample.                                                                                                                 | 50 µL/mL of sample                                                                                          | 50 µL/mL of sample                                                                                                                     |
|      | Mix and incubate.                                                                                                                            | RT for 3 minutes                                                                                            | RT for 3 minutes                                                                                                                       |
| 7    | Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                            | Top up to 2.5 mL                                                                                            | <ul style="list-style-type: none"> <li>• Top up to 3 mL for samples &lt; 2 mL</li> <li>• Top up to 10 mL for samples ≥ 2 mL</li> </ul> |
|      | Place the plasticware (without lid) into the magnet and incubate.                                                                            | RT for 10 minutes                                                                                           | RT for 10 minutes                                                                                                                      |
| 8    | Carefully pipette** (do not pour) off the supernatant. Remove the plasticware from the magnet; this plasticware contains the isolated cells. | Discard supernatant                                                                                         | Discard supernatant                                                                                                                    |
| 9    | Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.                            | Top up to 2.5 mL                                                                                            | <ul style="list-style-type: none"> <li>• Top up to 3 mL for samples &lt; 2 mL</li> <li>• Top up to 10 mL for samples ≥ 2 mL</li> </ul> |
|      | Place the plasticware (without lid) into the magnet and incubate.                                                                            | RT for 5 minutes                                                                                            | RT for 5 minutes                                                                                                                       |
| 10   | Carefully pipette** (do not pour) off the supernatant. Remove the plasticware from the magnet; this plasticware contains the isolated cells. | Discard supernatant                                                                                         | Discard supernatant                                                                                                                    |
| 11   | Repeat steps as indicated.                                                                                                                   | Steps 9 and 10<br>(total of 1 x 10 and 2 x 5-minute separations)                                            | Steps 9 and 10<br>(total of 1 x 10 and 2 x 5-minute separations)                                                                       |
| 12   | Resuspend cells in desired medium. Be sure to collect cells from the sides of the plasticware.                                               | Isolated cells are now ready for use                                                                        | Isolated cells are now ready for use                                                                                                   |

RT - room temperature (15 - 25°C)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

**Table 3. RoboSep™ Mouse CD4 Positive Selection Kit II Instructions**

| STEP | INSTRUCTIONS                                                                                                                                                                      | RoboSep™<br>(Catalog #20000 and #21000)                                                                                      |  |
|------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| 1    | Prepare sample at the indicated cell concentration within the volume range.                                                                                                       | 1 x 10e8 cells/mL<br>0.5 - 8 mL                                                                                              |                                                                                     |
|      | Add sample to required tube.                                                                                                                                                      | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning® Catalog #352057)                                         |                                                                                     |
| 2    | Add Normal Rat Serum to sample.                                                                                                                                                   | 50 µL/mL of sample                                                                                                           |                                                                                     |
| 3    | Prepare Selection Cocktail in the RoboSep™ Empty Vial provided. See Table 4 for required volumes.                                                                                 | Mix equal volumes of Component A and Component B (see Table 4).<br>Prepared cocktail is stable at 2 - 8°C for up to 4 weeks. |                                                                                     |
|      | Incubate.                                                                                                                                                                         | RT for 5 minutes                                                                                                             |                                                                                     |
| 4    | Select protocol.                                                                                                                                                                  | Mouse CD4 Positive Selection II 18952v2                                                                                      |                                                                                     |
| 5    | Vortex RapidSpheres™.                                                                                                                                                             | 30 seconds                                                                                                                   |                                                                                     |
| 6    | Load the carousel.                                                                                                                                                                | Follow on-screen prompts                                                                                                     |                                                                                     |
|      | Start the protocol.                                                                                                                                                               | Press the green “Run” button                                                                                                 |                                                                                     |
| 7    | Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube. | Isolated cells are now ready for use                                                                                         |                                                                                     |

**Table 4. RoboSep™ Selection Cocktail Preparation**

| START SAMPLE | COMPONENT A | COMPONENT B | SELECTION COCKTAIL<br>TOTAL VOLUME |
|--------------|-------------|-------------|------------------------------------|
| 0.5 mL       | 62.5 µL     | 62.5 µL     | 125 µL                             |
| 1 mL         | 75 µL       | 75 µL       | 150 µL                             |
| 1.5 mL       | 87.5 µL     | 87.5 µL     | 175 µL                             |
| 2 mL         | 100 µL      | 100 µL      | 200 µL                             |
| 3 mL         | 125 µL      | 125 µL      | 250 µL                             |
| 4 mL         | 150 µL      | 150 µL      | 300 µL                             |
| 5 mL         | 175 µL      | 175 µL      | 350 µL                             |
| 6 mL         | 200 µL      | 200 µL      | 400 µL                             |
| 7 mL         | 225 µL      | 225 µL      | 450 µL                             |
| 8 mL         | 250 µL      | 250 µL      | 500 µL                             |

Note: RoboSep™ requires an extra 100 µL of the Selection Cocktail to run properly (compared to manual protocols).

## Notes and Tips

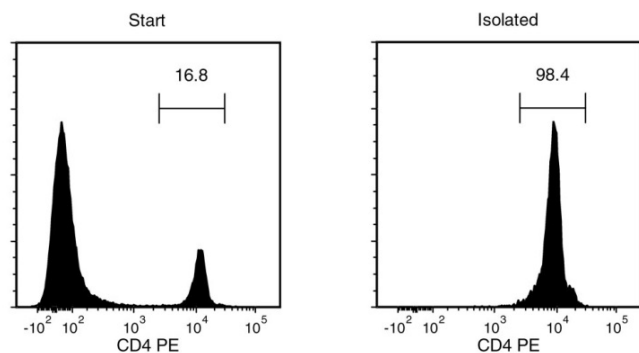
### ASSESSING PURITY

For purity assessment by flow cytometry use fluorochrome-conjugated:

- Anti-Mouse CD4 Antibody, Clone RM4-4 (Catalog #60029)

Other clones may be blocked and should be tested before use.

## Data



Starting with mouse splenocytes, the CD4+ cell content of the isolated fraction is typically  $98.6 \pm 0.4\%$  (mean  $\pm$  SD for the purple EasySep™ Magnet).

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