ALDHbr Assay Kit

For the detection of CD34+ and ALDHbr cells in human cord blood

Catalog #01711 For labeling 6 x 10^7 cells

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

The ALDHbr Assay Kit is used for the identification of human progenitor cells in cord blood that express CD34 and high levels of the enzyme aldehyde dehydrogenase (ALDH). It includes the ALDEFLUOR™ Kit and the CD34+ Cell Detection Kit. The CD34+ Cell Detection Kit contains pre-selected fluorochrome-conjugated monoclonal antibodies that have been optimized to be used with the ALDEFLUOR™ Kit to detect cells that express CD34 and have high ALDH activity by flow cytometry.

Product Information

The following kits are included in the ALDHbr Assay Kit.

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
ALDEFLUOR™ Kit*	01700	1 Kit	• Dry ALDEFLUOR™ Reagent • ALDEFLUOR™ Diethylaminobenzaldehyde (DEAB) Reagent, 1.5 mM in 95% ethanol • Hydrochloric Acid (HCl, 2 N) • Dimethylsulphoxide (DMSO) • ALDEFLUOR™ Assay Buffer
CD34+ Cell Detection Kit	01712	2 Kits	• 7-AAD Viability Dye • Anti-Human CD45 Antibody, Clone HI30, PE • Anti-Human CD34 Antibody, Clone 581, APC • Anti-Human CD235ab (Glycophorin A/B) Antibody, Clone HIR2, PE-Cyanine5

*Please refer to the Safety Data Sheet (SDS) for hazard information.

NOTE: For component details, refer to the ALDEFLUOR™ Kit Product information Sheet (PIS; Document #29888) or the CD34+ Cell Detection Kit PIS (Document #DX20507).

Materials Required But Not Included

A flow cytometer equipped with a 488 nm laser and a 635 nm laser for excitation and appropriate filters for detecting ALDEFLUOR™, 7-AAD, PE-Cyanine5, APC, and PE fluorescence is required.

Directions for Use

Please read the entire protocol before proceeding.

A) Cell Sample Preparation

1. Isolate cord blood mononuclear cells by standard density gradient centrifugation using Lymphoprep™ (Catalog #07801), HetaSep™ (Catalog #07906), Ficoll-Paque™ PLUS, or other density gradient medium. Resuspend cells in ALDEFLUOR™ Assay Buffer (Catalog #01701).

   NOTE: If using samples where the red blood cell (RBC) to leukocyte ratio (RBC:WBC) is > 1:1, deplete RBCs from the sample using ErythroClear™ Red Blood Cell Depletion Kit (Catalog #01739). Alternatively, RBCs may be depleted using reagents that do not contain detergents or fixatives (e.g. Ammonium Chloride Solution; Catalog #07800).
2. Adjust the sample to a concentration of 1 x 10^6 cells/mL with the ALDEFLUOR™ Assay Buffer.

B) Detection of 7-AAD-Glycophorin A/B-CD45+ALDHbrCD34+ Cells with the ALDEFLUOR™ Kit and the CD34+ Cell Detection Kit

I) ALDEFLUOR™ Kit

NOTE: Disregard the PIS included in the ALDEFLUOR™ Kit and use the full protocol below.

The dry ALDEFLUOR™ Reagent is provided in a stable, inactive form (BODIPY-aminoacetaldehyde-diethyl acetate, BAAA-DA). For use, the dry ALDEFLUOR™ Reagent is dissolved in DMSO, converted to the fluorescence-activated ALDEFLUOR™ Reagent (BAAA) by treatment with 2 N HCl and diluted with ALDEFLUOR™ Assay Buffer.

ALDEFLUOR™ Activation

1. Assemble all necessary supplies and allow kit reagents to come to room temperature (15 - 25°C) before use.
2. Add 25 µL of DMSO to the vial of dry ALDEFLUOR™ Reagent, mix well and let it stand for 1 minute at room temperature (15 - 25°C).

   NOTE: The dry ALDEFLUOR™ Reagent is an orange-red powder that changes to a bright yellow-green color upon addition of DMSO.
3. Add 25 µL of 2 N HCl and mix well. Incubate this mixture for 15 minutes at room temperature (15 - 25°C).

   NOTE: Adding 2 N HCl before DMSO will render the product inactive.
4. Add 360 µL of ALDEFLUOR™ Assay Buffer to the vial and mix.

   NOTE: Upon addition of the ALDEFLUOR™ Assay Buffer, the solution may appear slightly cloudy. This does not affect the assay performance.
5. Keep the activated ALDEFLUOR™ Reagent at 2 - 8°C during use.
6. Aliquot the remaining activated ALDEFLUOR™ Reagent and store at -20°C.

ALDEFLUOR™ Assay

1. For each sample to be tested, label one "test" and one "control" tube. Place 2 mL of the adjusted cell suspension into the "test" tube.
2. Add 5 µL of the ALDEFLUOR™ DEAB Reagent to the "control" tube. Recap the "control" tube and the DEAB vial immediately.
3. Add 10 µL of the activated ALDEFLUOR™ Reagent to the "test" tube containing the cell suspension. Mix well.
4. Immediately transfer 0.5 mL of the mixture to the "control" tube containing ALDEFLUOR™ DEAB Reagent. Mix well by pipetting up and down several times.
5. Incubate "test" and "control" samples for 30 minutes at 37°C, protected from light.
6. Place samples in an ice bath following incubation.

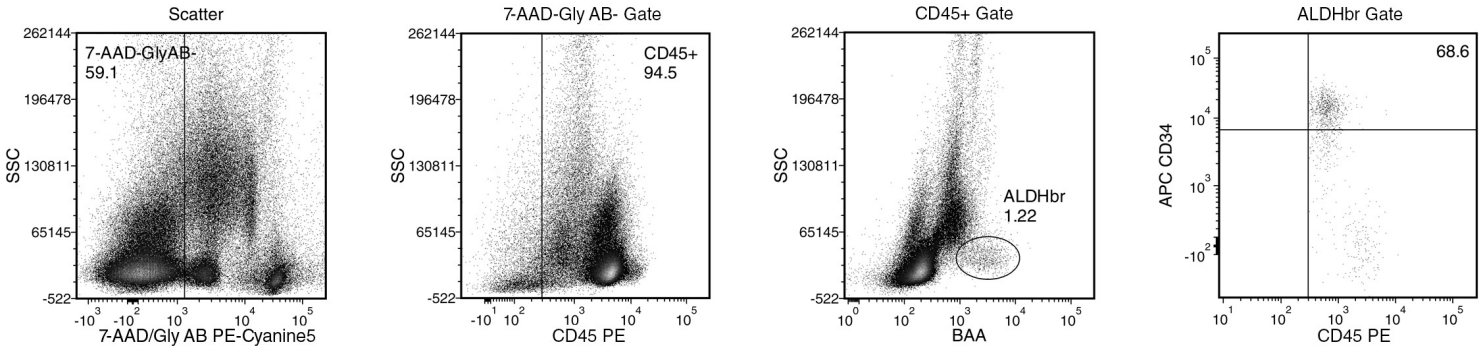
II) CD34+ Cell Detection Kit

7. Add 15 µL each of the Anti-Human CD34 APC, CD45 PE, and Glycophorin A/B PE-Cyanine5 Antibodies to the "test" tube.
8. Incubate for 15 minutes in an ice bath, protected from light.
9. Centrifuge both the "test" and the "control" tubes for 5 minutes at 250 x g. Discard the supernatant.
10. Wash both the “test” and the “control” tubes with ALDEFLUOR™ Assay Buffer.
11. Centrifuge both the "test" and the "control" tubes for 5 minutes at 250 x g. Discard the supernatant.
12. Resuspend the "test" cell pellet in 1.5 mL and the "control" cell pellet in 0.5 mL of ALDEFLUOR™ Assay Buffer.
13. Add 10 µL of the 7-AAD Viability Dye to the "test" tube and incubate for 5 - 10 minutes in an ice bath, protected from light.
14. Analyze on a flow cytometer equipped with a 488 nm laser and a 635 nm laser for excitation and appropriate filters for detecting ALDEFLUOR™, 7-AAD, PE-Cyanine5, APC, and PE fluorescence. Refer to the gating strategy in the Data Section for analysis of 7-AAD-Glycophorin A/B-CD45+ALDHbrCD34+ cells.

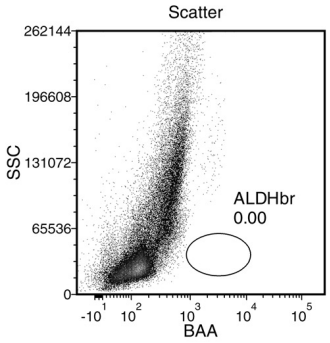
Notes and Tips

- For samples containing an RBC:WBC ratio of > 1:1, depletion of RBCs from the samples may be required prior to the assay. RBCs can be efficiently and effectively depleted using ErythroClear™ Red Blood Cell Depletion Kit (Catalog #01739) without affecting the frequency of cells with high ALDH activity and expressing CD34.
- When frozen aliquots of the activated ALDEFLUOR™ Reagent are thawed, a small precipitate (pellet) may be observed. Before use, mix the thawed reagent to suspend the precipitate. This precipitate does not affect assay performance.

Data



DEAB Control:



ALDHbr Assay Kit (7-AAD-Glycophorin A/B-CD45+ALDHbrCD34+ cells): Cord blood mononuclear cells (1 x 10^6 cells/mL) were stained and analyzed on a flow cytometer equipped with a 488 nm laser and a 635 nm laser for excitation and appropriate filters for detecting ALDEFLUOR™ (BAA), 7-AAD, PE-Cyanine5, APC, and PE fluorescence. The gating strategy for identifying 7-AAD-Glycophorin A/B-CD45+ALDHbrCD34+ cells is shown in the upper 4 plots above. The placement of the ALDHbr gate is verified using the DEAB control sample as shown in the lower single plot above.

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/cbworkflow or contact us at techsupport@stemcell.com.