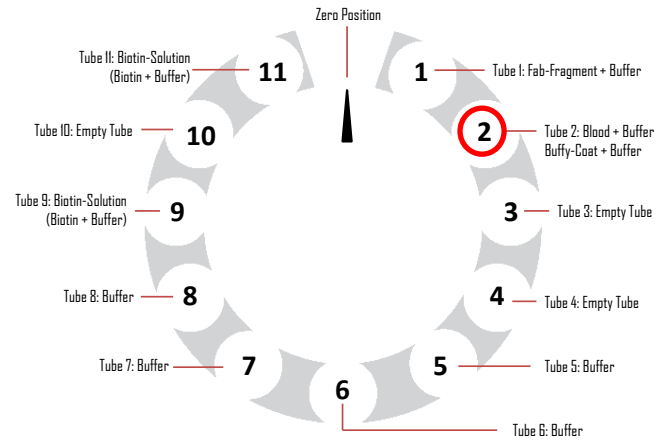


Isolation Kit for CD28+ cells from Whole Blood (human) or Buffy Coat (human) with FABian®

Order Number: 6-6014-001

1 General Information

- Intended use:** This kit is for research use only.
- Capacity:** For up to 9 ml whole blood with anticoagulants (e.g. heparin) or up to 6.25 ml buffy coat
- Components:**
- 1x set consisting of 10 ml hub syringe, 11 tubes (30 ml) and a luer lock connection (#6-6999-006)
 - 1x column filled with cell grade agarose, 0.5 ml (#6-6998-500)
 - 1x vial CD28 Fab-Strep, human, lyophilized (#6-8014-090)
 - 1x vial 100 mM Biotin stock solution for the elution of cells, 250 µl (#6-6996-001)
 - 1x bottle of buffer CI for cell isolation with Fabian®, 100 ml (#6-6997-100)
- Not included are serological pipettes, pipette tips, microliter pipettes and antibodies for FACS Analysis of isolated cells.
- Storage:** Store Fab-Strep, Buffer CI, Biotin and the TACS Auto column at +4° C to +8° C.
- Expiration dates are indicated on the labels of the products.



3. Add to 9 ml whole blood 3.5 ml of Buffer CI. Alternatively add 6.25 ml buffy coat to 6.25 ml of Buffer CI. Mix gently by pipetting up and down and pipette 12.5 ml in tube no. 2 (please see figure above and the table below).
4. Prepare Biotin elution buffer by adding 250 µl of 100 mM Biotin solution to 25 ml Buffer CI and mix with vortex.
5. Distribute by pipetting 10 ml of this Biotin-elution buffer to tube no. 9 and 15 ml to tube no. 11.
6. According to the table below pipette Buffer CI to tubes no. 5, 6, 7 and 8.
7. Pipette 3 ml Buffer CI into tube no. 1.
8. Dissolve the CD28 Fab-Strep in 600 µl Buffer CI by pipetting and add it to the 3 ml of Buffer CI in tube no. 1.
9. Rinse the FAB vial with additional 600 µl of Buffer CI and add it to tube no. 1. Mix thoroughly.

2 Protocol

Preparation of the reagents

Adjust all reagents and materials to room temperature (19-25°C) before use.

1. Collect up to 9 ml whole blood with anticoagulant (Heparin) from a donor. Alternatively the kit can be used for up to 6.25 ml buffy coat.
2. Place the 30 ml tubes without lid in the tube holder of the FABian®. For a sterile isolation process place the FABian® in a safety cabinet.

Position	Content	Standard	Volume
1	FAB in Buffer CI	4.2 ml	ml
2	Whole blood+Buffer CI buffy coat+Buffer CI	9 ml + 3.5 ml 6.25 ml + 6.25 ml	ml
3	Empty tube		ml
4	Empty tube		ml
5	Buffer CI	15 ml	ml
6	Buffer CI	10 ml	ml
7	Buffer CI	10 ml	ml
8	Buffer CI	5 ml	ml
9	Biotin Solution	10 ml	ml
10	Empty tube		ml
11	Biotin Solution	15 ml	ml

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Cell Selection with FABian®

For operation of FABian® read the instruction manual of the device carefully. For insertion of the syringe and starting of the program proceed according to the FABian® manual.

Completion of hub syringe and column:

1. The column is delivered in a screw cap tube with a buffer supernatant. Remove the colored plug from the column; pull out the plunger of the included syringe (10 ml BBraun Omnifix®) just enough to allow the removal of the buffer supernatant. Attach the syringe to the column using the white adapter and gently squeeze the buffer supernatant out of the column. Avoid letting the matrix run dry or trapping air in the column.
2. The column with the syringe can now be inserted in the lid of FABian®.
3. For this step see instruction manual of FABian®.

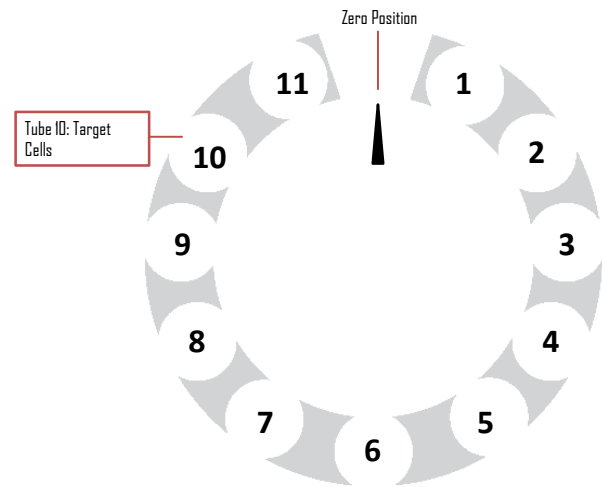


Selecting the program:

1. The isolation of CD28+ cells can now be performed by using the standard program provided by IBA GmbH. Sign up on the Download area of our homepage www.iba-lifesciences.com/download-area and select the program called **hCD28 short program** (53 min; suited for 4.5 ml whole blood and buffy coat each) or **hCD28 long program** (87 min; suited for 9 ml whole blood and 6,25 ml buffy coat).
2. Store the *.txt file on the supplied SD card under the file name 1,2, A or B. This determines under which of the FABian® keys the program is accessible.

Running the program:

1. Now insert the SD card, press the button 1, 2, A or B according to the stored file name and press GO (please also refer to the FABian® manual).
2. The program now runs automatically till its end. The selected target cells can be taken from tube 10, as shown in the following figure.

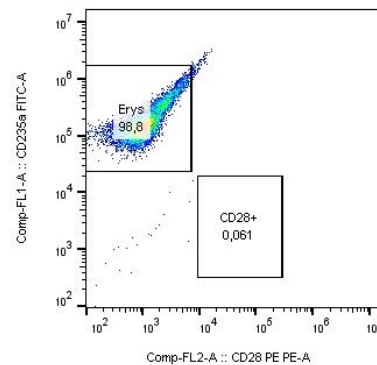


3 Example Analysis

Isolation of CD28+ cells from buffy coat with FABian®

CD28+ cells were isolated from 6.25 ml buffy coat with the automated system FABian® using the Isolation Kit for CD28+ cells (order no.: 6-6014-001). CD28+ cells were separated in high yields and excellent purity (> 90%) from the blood sample.

Before separation of CD28+ cells



After separation of CD28+ cells

