cFluor® Anti-Human CD2 (RPA-2.10)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11143	100	cFluor® R720 Anti-Human CD2 (RPA-2.10)
R7-11144	25	cFluor® R720 Anti-Human CD2 (RPA-2.10)

Cytek Biosciences, Inc
47215 Lakeview Blvd.
Fremont, CA 94538
USA
1.877.92.CYTEK (1.877.922.9835)

products@cytekbio.com
cytekbio.com

EC REP

Emergo Europe
Prinsessegracht 20
2514 AP The Hague
Netherlands

R9-51002US Rev. A
April 2022

CE IVD

1. Intended use

This product is intended for in vitro diagnostic use to identify human cells expressing CD2 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The CD2 (RPA-2.10) monoclonal antibody binds to human CD2, a 50-kDa type I transmembrane glycoprotein. CD2, also known as LFA-2, T11, and sheep red blood cell receptor (SRBC-R), is expressed on thymocytes, T lymphocytes, NK cells, and thymic B cell subsets. The major ligand for CD2 is CD58 (also known as LFA-3). CD2 has also been reported to bind CD48, CD59, and CD15. CD2 plays a role in alternative T cell activation, T cell signaling, and cell-cell adhesion. The antibody is conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD2 monoclonal antibody conjugated with the following listed cFluor fluorescent dye is supplied in phosphate-buffered saline, pH 7.2, containing 0.09% sodium azide and 0.2% BSA (BSA Country of Origin USA).

Antibody specificity	CD2
Clone	RPA-2.10
Immunoglobulin subtype	IgG1, kappa
Species and genus	Mouse
Fluorescent dye	cFluor® R720[1]
Excitation wavelength	640 nm
Emission peak	720 nm

4. Storage and Handling

This product is stable until the expiration date shown on the label when stored away from light at 2 ~ 8 °C. Do not freeze.

5. Other Materials required but not supplied

- RBC lysing solution
- Pipettes and pipette tips of 20 µL, 100 µL and 1000 µL
- 12x75mm tube
- Vortex mixer
- Flow cytometer

6. Specimen Requirements

1 Require peripheral blood of not less than 500 µL collected by venipuncture in EDTA anticoagulation tube.

2 After collection, the samples should be stored at room temperature (18 ~ 25 ℃). Avoid shaking. The storage time should not exceed 24 hours.

3 After staining, the samples should be stored at 2 ~ 8 ℃ away from light and analyzed by flow cytometry within 2 hours.

4 Avoid samples with microbial contamination or coagulation.

7. Procedure

1 Add 100 µL well-mixed EDTA anticoagulated whole blood to the bottom of a tube. Avoid blood touching the upper tube wall.

2 Briefly centrifuge this product before use. Add 5 µL of CD2-cFluor-conjugated reagent to the bottom of the tube.

3 Mix well by vortex and incubate for 15-30 minutes at room temperature and away from light.

4 Add 2 mL of 1 X lysis buffer into the tube, mix briefly by vortex, and incubate for 10-15 minutes at room temperature in the dark.

5 Centrifuge at 300g for 5 minutes, discard the supernatant, add 2 mL PBS with 0.02% BSA, and 0.09% NaN_3 to resuspend the cell.

6 Centrifuge at 300g for 5 minutes, discard the supernatant, add 300 µL PBS with 0.02% BSA, and 0.09% NaN_3 to resuspend the cells and keep at 4 ℃, and analyze on flow cytometer within 2 hours. If delayed analysis is needed (more than 2 hours), 300 µL of PBS containing 1% paraformaldehyde should be used to resuspend the cells and store the sample in a refrigerator at 2-8 ℃ away from light, but the storage time should not exceed 24 hours.

8. Quality Control

- Instrument QC: Use the manufacturer recommended controls according to the model of the flow cytometer.
- Refer to the instrument User's Guide for instrument maintenance.

9. Warnings

- This reagent contains traces of sodium azide. Do not pipette by mouth.
- Use appropriate personal protective equipment per the safety data sheet when using this product.
- Follow biosafety practice in compliance with federal, state, and local regulations to handle all biological samples and materials in contact with them.
- Contact Cytek Support or refer to cytekbio.com for details on troubleshooting.

10. Performance Characteristics

10.1. Accuracy

Three replicate tubes were stained with CD2-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD2+ lymphocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	**Percent CD2+ Lymphocytes**				
CD2-Fluorescent Dye	R1	R2	R3	Mean	Target Value Range
cFluor R720	85.3	85.7	85.6	85.5	73.5-97.5

10.2. Intra-batch precision

Ten replicate tubes were stained with the same batch of CD2-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD2+ lymphocytes was calculated and was within the acceptance criteria.

Specimen: Normal Blood	**Percent CD2+ Lymphocytes**		
CD2-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor R720	85.6	0.42	CV≤8%

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of CD2-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD2+ lymphocytes was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD2+ Lymphocytes**		
CD2-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor R720	85.4	0.38	CV≤8%

10.4. Staining stability

Three replicate tubes were stained with the same batch of CD2-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer at these timepoints: within 2-hour (T0), 24-hour, and 48-hour after staining. The percent CD2+ lymphocytes at each time point were compared to T0, and the mean relative difference was calculated and was within the acceptance criteria.

Specimen: Normal Blood	**Percent CD2+ Lymphocytes**			
CD2-Fluorescent Dye	Average (%)	Relative Difference vs. 2H		Criteria
		24H	48H	
cFluor R720	85.5	0.77%	0.41%	Relative Difference ≤10%

11. Limitations

1. This reagent can be used with a flow cytometer and is not recommended for fluorescence microscopy and immunohistochemistry.
2. This reagent is a fluorescent labeled product. It is easy to quench with extended light exposure and should be handled away from light.
3. If not following the lyse wash procedure described above, the reagent performance can be affected.
4. The results may be affected by improper storage of reagents, coagulation of specimens, improper storage of specimens, or incomplete lysis of red blood cells in the samples.
5. The test results of this reagent are for clinical reference only. Patient history, other laboratory tests, and treatment response should also be considered for diagnosis.

12. References

- Moingeon P, et al. 1989. Immunol Rev. 111: 111-44
- Schraven B. J Biol Regul Homeost Agents. 14(3):223-5
- Binder C, et al. Front Immunol. 11: 1090

[1]cFluor® R720 is equivalent to CF®700 manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytek (LICENSEE). The manufacture, use, sale, offer for sale, or import of the product is covered by one or more of the patents or pending applications owned or licensed by Biotium. The purchase of this product includes a limited, nontransferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.