



cFluor® Anti-Human CD4 (SK3)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11027	100	cFluor® B515 Anti-Human CD4 (SK3)
R7-11028	25	cFluor® B515 Anti-Human CD4 (SK3)
R7-11037	100	cFluor® B532 Anti-Human CD4 (SK3)
R7-11038	25	cFluor® B532 Anti-Human CD4 (SK3)
R7-11043	100	cFluor® B548 Anti-Human CD4 (SK3)
R7-11044	25	cFluor® B548 Anti-Human CD4 (SK3)
R7-11049	100	cFluor® R668 Anti-Human CD4 (SK3)
R7-11050	25	cFluor® R668 Anti-Human CD4 (SK3)
R7-11045	100	cFluor® R720 Anti-Human CD4 (SK3)
R7-11046	25	cFluor® R720 Anti-Human CD4 (SK3)
R7-11057	100	cFluor® V450 Anti-Human CD4 (SK3)
R7-11058	25	cFluor® V450 Anti-Human CD4 (SK3)
R7-11003	100	cFluor® BYG781 Anti-Human CD4 (SK3)
R7-11004	25	cFluor® BYG781 Anti-Human CD4 (SK3)

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1. Intended Use

This product is intended for in vitro diagnostic use to identify human cells expressing CD4 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The CD4 (SK3) monoclonal antibody binds to human CD4, a 59-kDa type I transmembrane glycoprotein in the immunoglobulin superfamily. The CD4 molecule is expressed predominantly on thymocytes and a subpopulation of mature T-helper lymphocytes. It is also present on monocytes at low levels. CD4 plays a role in cell-cell interaction by acting as a co-receptor for MHC class II in antigen recognition. CD4 also has been shown to bind glycoprotein 120 on the external envelope of HIV. The antibody is conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD4 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	CD4	CD4	CD4	CD4	CD4	CD4	CD4
Clone	SK3	SK3	SK3	SK3	SK3	SK3	SK3
Immunoglobulin subtype	IgG1, kappa	IgG1, kappa	IgG1, kappa	IgG1, kappa	IgG1, kappa	IgG1, kappa	IgG1, kappa
Species and genus	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse	Mouse
Fluorescent dye	cFluor [®] B515 ¹	cFluor [®] B532 ¹	cFluor [®] B548 ¹	cFluor [®] R668 ¹	cFluor [®] R720 ¹	cFluor [®] V450	cFluor [®] BYG781 ²
Excitation wavelength	488 nm	488 nm	488 nm	640 nm	640 nm	405 nm	488 nm
Emission peak	515 nm	532 nm	548 nm	668 nm	720 nm	450 nm	781 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 $^{\circ}\text{C}$). Avoid shaking. The storage time should not exceed 24 hours.
- 3 After staining, the samples should be stored at 2 ~ 8 $^{\circ}\text{C}$ 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 CD4-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 $^{\circ}\text{C}$, 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 $^{\circ}\text{C}$ 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 Cytex Support or refer to cytekbio.com for details on troubleshooting.

10. Performance Characteristics

10.1. Accuracy

Three replicate tubes were stained with each CD4-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD4+ T lymphocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	Percent CD4+ T Lymphocytes				
CD4-Fluorescent Dye	R1	R2	R3	Mean	Target Value Range
cFluor B515	49.2	48.8	48.5	48.9	42.7-54.7
cFluor B532	47.6	48.9	48.2	48.3	42.7-54.7
cFluor B548	45.6	45.0	47.9	46.2	42.7-54.7
cFluor BYG781	50.6	51.6	50.8	51.0	44.4-56.4
cFluor R668	48.6	47.8	49.3	48.5	42.7-54.7
cFluor R720	48.4	49.1	47.5	48.4	42.7-54.7
cFluor V450	46.8	49.5	46.7	47.7	42.7-54.7

10.2. Intra-batch Precision

Ten replicate tubes were stained with the same batch of each CD4-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD4+ T lymphocytes were calculated and were within the acceptance criteria.

Specimen: Normal Blood	Percent CD4+ T Lymphocytes		
CD4-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor B515	25.4	3.02	CV≤15%
cFluor B532	46.9	1.07	CV≤8%
cFluor B548	46.8	1.34	
cFluor BYG781	48.2	1.64	
cFluor R668	48.2	1.40	
cFluor R720	47.3	1.27	
cFluor V450	50.3	1.60	

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of each CD4-cFluor-conjugated reagent and analyzed on Cytex Northern Lights™ flow cytometer. The CV of percent CD4+ T lymphocytes were calculated and were within the acceptance criteria.

Specimen: CD-CHEX PLUS	Percent CD4+ T Lymphocytes		
CD4-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor B515	43.3	5.37	CV≤8%
cFluor B532	46.7	1.19	
cFluor B548	47.5	1.15	
cFluor BYG781	46.8	1.66	
cFluor R668	48.9	1.76	
cFluor R720	47.5	2.00	
cFluor V450	49.9	1.37	

10.4. Staining stability

Three replicate tubes were stained with the same batch of each CD4-cFluor-conjugated reagent and analyzed on Cytex Northern Lights™ flow cytometer at these timepoints: within 2-hour (T0), 6-hour, 24-hour, 48-hour, 72-hour after staining. The percent CD4+ T 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 CD4+ T Lymphocytes					
CD4-Fluorescent Dye	Average (%)	Relative Difference vs. 2H				Criteria
		6H	24H	48H	72H	
cFluor B515	48.3	0.14%	1.75%	2.83%	3.22%	Relative Difference ≤10%
cFluor B532	47.0	0.15%	0.99%	2.05%	3.01%	
cFluor B548	47.1	1.10%	0.04%	0.52%	0.66%	
cFluor BYG781	48.2	-1.05%	1.79%	0.14%	NA	
cFluor R668	46.2	4.46%	5.64%	6.89%	7.28%	
cFluor R720	47.4	2.05%	3.55%	0.58%	0.33%	
cFluor V450	47.6	-0.57%	3.17%	3.57%	3.94%	

10.5. Dilution linearity

The samples were serially diluted into five levels (undiluted, 2X, 4X, 8X, 16X). Four replicate tubes at each dilution level were stained with the same batch of each CD4-cFluor-conjugated reagent and analyzed on Cytex Northern Lights™ flow cytometer. The median of percent CD4+ T lymphocytes

at each dilution level were compared to the median of percent CD4+ T lymphocytes at all levels, the relative difference was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	Percent CD4+ T Lymphocytes					
CD4-Fluorescent Dye	Relative Difference vs. 2H					Criteria
	Undiluted	2X dilution	4X dilution	8X dilution	16X dilution	
cFluor B515	1.50%	-0.37%	1.78%	-1.08%	-3.10%	Relative Difference ≤10%
cFluor B532	0.79%	0.00%	0.80%	-0.09%	-0.13%	
cFluor B548	0.73%	0.18%	0.52%	0.66%	-1.37%	
cFluor BYG781	0.69%	1.57%	1.29%	-1.98%	-0.71%	
cFluor R668	-2.74%	0.34%	-0.36%	1.40%	1.22%	
cFluor R720	1.45%	1.17%	0.25%	-0.86%	-2.42%	
cFluor V450	1.62%	0.78%	0.53%	-0.53%	-3.89%	

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, 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

- Evans RL, et al. 1981. Immunol. 78:544
- Muench M, et al. 1997. Blood 89:1364
- Arno A et al. 1999. J. Infect. Dis. 180:56

¹cFluor[®] B515, cFluor[®] B532, cFluor[®] B548, cFluor[®] R668, and cFluor[®] R720 are equivalent to CF[®]488A, CF[®]503R, CF[®]514, CF[®]647, and CF[®]700 respectively, manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytex (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.

²cFluor[®] BYG781 is a tandem dye made with R-PE. Caution: Tandem dyes may show changes in their emission spectra with prolonged exposure to light or fixatives.