cFluor® Anti-Human CD3 (SK7)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11001	100	cFluor® B520 Anti-Human CD3 (SK7)
R7-11002	25	cFluor® B520 Anti-Human CD3 (SK7)
R7-11053	100	cFluor® V420 Anti-Human CD3 (SK7)
R7-11054	25	cFluor® V420 Anti-Human CD3 (SK7)

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CE IVD

1. Intended Use

This product is intended for in vitro diagnostic use to identify human cells expressing CD3 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The SK7 monoclonal antibody binds to human epsilon chain of the CD3 antigen/T-cell antigen receptor, a 20 to 30-kDa protein complex. This complex contains a CD3γ, a CD3δ, a CD3ζ (CD247), two CD3ε, and a T-cell receptor (αβ or γδ) heterodimer. CD3 is found on all mature T cells, NK T cells, and some thymocytes. It plays a role in recognizing antigen, activating cytotoxic T cell and T helper cell, and signal transduction. The antibody is conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD3 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	**CD3**	**CD3**
Clone	SK7	SK7
Immunoglobulin subtype	IgG1, kappa	IgG1, kappa
Species and genus	Mouse	Mouse
Fluorescent dye	cFluor® B520	cFluor® V420
Excitation wavelength	488 nm	405 nm
Emission peak	520 nm	420 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 ℃ . 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 CD3-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 each CD3-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD3+ lymphocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	**Percent CD3+ Lymphocytes**				
CD3-Fluorescent Dye	**R1**	**R2**	**R3**	**Mean**	**Target Value Range**
cFluor B520	77.3	77.5	76.7	77.2	67.5-87.5
cFluor V420	76.9	76.8	78.6	77.5	66.3-86.3

10.2. Intra-batch Precision

Ten replicate tubes were stained with the same batch of each CD3-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD3+ lymphocytes was calculated and was within the acceptance criteria.

Specimen: Normal Blood	**Percent CD3+ Lymphocytes**		
CD3-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor B520B	74.9	0.65	CV≤8%
cFluor V420	61.6	0.74	

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of each CD3-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD3+ lymphocytes was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD3+ Lymphocytes**		
CD3-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor B520	75.1	1.21	CV≤8%
cFluor V420	66.0	1.02	

10.4. Staining stability

Three replicate tubes were stained with the same batch of each CD3-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer at these timepoints: within 2-hour (T0), 6-hour, 24-hour, 48-hour, and 72-hour (for B520 only) after staining. The percent CD3+ 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 CD3+ Lymphocytes**					
CD3-Fluorescent Dye	Average (%)	Relative Difference vs. 2H				Criteria
		6H	24H	48H	72H	
cFluor B520	75.1	-0.83%	0.01%	-0.36%	NA	Relative Difference ≤10%
cFluor V420	65.4	0.15%	0.73%	1.64%	0.42%	

10.5. Dilution linearity

The samples were serial diluted into five levels (undiluted, 2X, 4X, 8X, 16X). Four replicate tubes at each dilution level were stained with the same batch of each CD3-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The median of percent CD3+ lymphocytes at each dilution level were compared to the median of percent CD3+ lymphocytes at all levels, and the relative difference was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD3+ Lymphocytes**					
CD3-Fluorescent Dye	Relative Difference vs. 2H					Criteria
	Undiluted	2X dilution	4X dilution	8X dilution	16X dilution	
cFluor B520	0.37%	0.19%	0.67%	-0.33%	-0.61%	Relative Difference ≤10%
cFluor V420	0.12%	0.00%	0.59%	1.03%	0.07%	

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

- Dong, D., et al. 1981. Nature. 573, 546–552
- Weiss A, et al. 1991. Semin Immunol. (5):313-24