



cFluor[®] Anti-Human CD15 (HI98)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11025	100	cFluor [®] B548 Anti-Human CD15 (HI98)
R7-11026	25	cFluor [®] B548 Anti-Human CD15 (HI98)

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1. Intended use

This product is intended for in vitro diagnostic use to identify human cells expressing CD15 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The HI98 monoclonal antibody binds to human CD15, a 220-kDa carbohydrate structure. CD15 is also known as 3-fucosyl-N-acetylactosamine (3-FAL), Lewis X, 3-FAL, X-hapten, and SSEA-1. CD15 is highly expressed on granulocytes, including neutrophils and eosinophils, and some on monocytes. It is absent from lymphocytes or basophils. CD15, a marker for human myeloid cell, is involved in various cell functions including phagocytosis, bacterial activity, neutrophil adhesion to dendritic cells, and chemotaxis. The antibody is conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD15 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	CD15
Clone	HI98
Immunoglobulin subtype	IgM, kappa
Species and genus	Mouse
Fluorescent dye	cFluor [®] B548 ¹
Excitation wavelength	488 nm
Emission peak	548 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 °C). Avoid shaking. The storage time should not exceed 24 hours.
- 3 After staining, the samples should be stored at 2 ~ 8 °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 CD15-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% NaN₃ 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% NaN₃ to resuspend the cells and keep at 4 °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 °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 CD15-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD15+ granulocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	Percent CD15+ Granulocytes				
CD15-Fluorescent Dye	R1	R2	R3	Mean	Target Value Range
cFluor B548	99.7	99.6	99.7	99.7	90-100

10.2. Intra-batch precision

Ten replicate tubes were stained with the same batch of CD15-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD15+ granulocytes was calculated and was within the acceptance criteria.

Specimen: Normal Blood	Percent CD15+ Granulocytes		
CD15-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor B548	51.9	1.86	CV≤8%

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of CD15-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD15+ granulocytes was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	Percent CD15+ Granulocytes		
CD15-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor B548	51.5	2.05	CV≤8%

10.4. Staining stability

Three replicate tubes were stained with the same batch of CD15-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 after staining. The percent CD15+ granulocytes 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 CD15+ Granulocytes					
CD15-Fluorescent Dye	Average (%)	Relative Difference vs. 2H				Criteria
		6H	24H	48H	72H	
cFluor B548	41.8	-0.83%	-0.02%	0.60%	0.43%	Relative Difference ≤10%

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 CD15-cFluor-conjugated reagent and analyzed on Cytex Northern Lights™ flow cytometer. The median of percent CD15+ granulocytes at each dilution level were compared to the median of percent CD15+ granulocytes at all levels, the relative difference was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	Percent CD15+ Granulocytes					
CD15-Fluorescent Dye	Relative Difference vs. 2H					Criteria
	Undiluted	2X dilution	4X dilution	8X dilution	16X dilution	
cFluor B548	2.99%	-0.37%	-0.13%	-0.98%	0.26%	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

- Gadhoum SZ, et al. 2008. Nat Chem Biol. 4(12):751-757.
- Lund-Johansen F, et al. 1992. J Immunol. 148(10):3221-3229.

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