



cFluor[®] Anti-Human CD33 (WM53)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11021	100	cFluor [®] BYG610 Anti-Human CD33 (WM53)
R7-11022	25	cFluor [®] BYG610 Anti-Human CD33 (WM53)

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1. Intended use

This product is intended for in vitro diagnostic use to identify human cells expressing CD33 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The WM3 monoclonal antibody binds to human CD33, a 67-kDa type I transmembrane glycoprotein in the immunoglobulin family. CD33, also known as Siglec-3, gp67, and p67, is expressed on monocytes, activated T cells, myeloid progenitors, granulocytes, dendritic cells, and mast cells. It is absent on erythrocytes, platelets, and lymphoid cells. CD33 binds to sialic acids to act as a sialic acid-dependent cell adhesion molecule. It also contains immunoreceptor tyrosine-based inhibition motifs (ITIMs), suggesting its function to inhibit cellular activity. The antibody was conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD33 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	CD33
Clone	WM53
Immunoglobulin subtype	IgG1, kappa
Species and genus	Mouse
Fluorescent dye	cFluor [®] BYG610 ¹
Excitation wavelength	488 nm
Emission peak	610 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 CD33-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 CD33-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD33+ monocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	Percent CD33+ Monocytes				
CD33-Fluorescent Dye	R1	R2	R3	Mean	Target Value Range
cFluor BYG610	17.1	16.3	16.6	16.7	12.9-19.3

10.2. Intra-batch precision

Ten replicate tubes were stained with the same batch of CD33-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD33+ monocytes was calculated and was within the acceptance criteria.

Specimen: Normal Blood	Percent CD33+ Monocytes		
CD33-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor BYG610	18.2%	6.34%	CV≤15%

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of CD33-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD33+ monocytes was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	Percent CD33+ Monocytes		
CD33-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor BYG610	18.4	4.49%	CV≤15%

10.4. Staining stability

Three replicate tubes were stained with the same batch of CD33-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 CD33+ monocytes 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 CD33+ Monocytes		
CD33-Fluorescent Dye	Average (%)	Relative Difference vs. 2H	
		24H	48H
cFluor BYG610	17.4	8.12%	4.66%
Criteria: Relative Difference ≤20%			

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

- Favaloro EJ, et al. 1987. Dis Markers. 5(4):215-225
- Nakamura Y, et al. 1994. Blood. 83(5):1442-3
- Freeman SD, et al. 1995. Blood. 85(8):2005-2012
- Walter RB, et al. 2007. Traffic. 9(2):267-279

¹cFluor[®] BYG610 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.