cFluor® Anti-Human CD16 (3G8)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11069	100	cFluor® R668 Anti-Human CD16 (3G8)
R7-11070	25	cFluor® R668 Anti-Human CD16 (3G8)
R7-11075	100	cFluor® R720 Anti-Human CD16 (3G8)
R7-11076	25	cFluor® R720 Anti-Human CD16 (3G8)
R7-11007	100	cFluor® BYG575 Anti-Human CD16 (3G8)
R7-11008	25	cFluor® BYG575 Anti-Human CD16 (3G8)

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

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CE IVD

1. Intended Use

This product is intended for in vitro diagnostic use to identify human cells expressing CD16 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The 3G8 monoclonal antibody binds to IgG receptor III (FcγRIII) that are in two forms: CD16a (FcγRIIIA) and CD16b (FcγRIIIB). With 95% sequence similarity, they are a conventional 50-65 kDa polypeptide-anchored transmembrane protein and a 48 kDa GPI-anchored protein, respectively. CD16a is expressed on NK cells and macrophages while CD16b is expressed on neutrophils. CD16a also plays a crucial role for antibody-dependent cellular cytotoxicity (ADCC) by NK cells. The antibody is conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD16 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	**CD16**	**CD16**	**CD16**
Clone	3G8	3G8	3G8
Immunoglobulin subtype	IgG1, kappa	IgG1, kappa	IgG1, kappa
Species and genus	Mouse	Mouse	Mouse
Fluorescent dye	cFluor® R668[1]	cFluor® R720[1]	cFluor® BYG575
Excitation wavelength	630 nm	630 nm	488 nm
Emission peak	668 nm	720 nm	575 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 CD16-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 CD16-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD16+ lymphocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	**Percent CD16+ Lymphocytes**				
CD16-Fluorescent Dye	**R1**	**R2**	**R3**	**Mean**	**Target Value Range**
cFluor R668	9.0	9.9	9.8	9.6	5-15
cFluor R720	9.4	8.9	9.9	9.4	
cFluor BYG575	10.9	11.1	12.2	11.4	

10.2. Intra-batch Precision

Ten replicate tubes were stained with the same batch of each CD16-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD16+ lymphocytes was calculated and was within the acceptance criteria.

Specimen: Normal Blood	**Percent CD16+ Lymphocytes**		
CD16-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor R668	25.8	4.20	CV≤15%
cFluor R720	8.4	5.14	
cFluor BYG575	10.2	4.40	

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of each CD16-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD16+ lymphocytes was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD16+ Lymphocytes**		
CD16-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor R668	24.5	6.19	CV≤15%
cFluor R720	7.6	6.21	
cFluor BYG575	12.4	2.98	

10.4. Staining stability

Three replicate tubes were stained with the same batch of each CD16-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer at these timepoints: within 2-hour (T0), 6-hour, 24-hour, 48-hour, 72-hour (excluding BYG575) after staining. The percent CD16+ 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 CD16+ Lymphocytes**					
CD16-Fluorescent Dye	Average (%)	Relative Difference vs. 2H				Criteria
		6H	24H	48H	72H	
cFluor R668	8.7	-5.16%	1.16%	1.85%	-1.16%	Relative Difference ≤30%
cFluor R720	7.0	-4.30%	0.19%	3.87%	-1.43%	
cFluor BYG575	10.0	3.98%	-2.11%	-2.64%	NA	Relative Difference ≤20%

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 CD16-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The median of percent CD16+ lymphocytes at each dilution level were compared to the median of percent CD16+ lymphocytes at all levels. The relative difference was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD16+ Lymphocytes**					
CD16-Fluorescent Dye	Relative Difference vs. 2H					Criteria
	Undiluted	2X dilution	4X dilution	8X dilution	16X dilution	
cFluor R668	0.42%	0.31%	-0.20%	-0.33%	-0.46%	Relative Difference ≤3%
cFluor R720	1.16%	-0.47%	-0.08%	-0.54%	0.81%	
cFluor BYG575	-0.06%	0.04%	0.16%	-0.07%	0.01%	

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

- Wirthmueller U, et al. 1992. J Exp Med. 175:1381
- Smed-Sörensen A, et al. 2008. Blood. 111:5037
- Wei H Y, et al, 2016. Sci Rep. 6:34310

[1]cFluor® R668 and cFluor® R720 are equivalent to CF®647, and CF®700 respectively, 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, non-transferable 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.