cFluor® Anti-Human CD14 (M5E2)

Instructions For Use

Catalog No.	Test/Vial	Product Name
R7-11077	100	cFluor® V450 Anti-Human CD14 (M5E2)
R7-11078	25	cFluor® V450 Anti-Human CD14 (M5E2)

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CE IVD

1. Intended use

This product is intended for in vitro diagnostic use to identify human cells expressing CD14 antigen molecules in countries where the regulatory approval has been obtained from the local regulatory authorities.

2. Application

The M5E2 monoclonal antibody binds to human CD14, a 53-55-kDa glycosylphosphatidylinositol (GPI)-linked membrane glycoprotein that works as a receptor on myeloid cells for ligands such as lipopolysaccharide (LPS). It is found that CD14 is a receptor for and binds to complexes of LPS and LBP with high affinity. It expresses on monocytes and macrophages at high levels. It is also present in some interfollicular dendritic cells and macrophages, reticular dendritic cells, and Langerhans cells. The antibody is conjugated to a fluorophore and purified by affinity chromatography.

3. Components

CD14 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	CD14
Clone	M5E2
Immunoglobulin subtype	IgG2α, kappa
Species and genus	Mouse
Fluorescent dye	cFluor® V450
Excitation wavelength	405 nm
Emission peak	450 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 ℃). 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 CD14-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 CD14-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The percent CD14+ monocytes results were within the control blood target value range provided by the manufacturer.

Specimen: CD-CHEX PLUS	**Percent CD14+ Monocytes**				
CD14-Fluorescent Dye	R1	R2	R3	Mean	Target Value Range
cFluor V450	90.4	92.0	92.5	91.6	70-100

10.2. Intra-batch precision

Ten replicate tubes were stained with the same batch of CD14-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD14+ monocytes was calculated and was within the acceptance criteria.

Specimen: Normal Blood	**Percent CD14+ Monocytes**		
CD14-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor V450	80.2	1.08%	CV≤8%

10.3. Inter-batch precision

Three replicate tubes were stained with three batches of CD14-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The CV of percent CD14+ monocytes was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD14+ Monocytes**		
CD14-Fluorescent Dye	Average (%)	% CV	Criteria
cFluor V450	80.6	1.16%	CV≤8%

10.4. Staining stability

Three replicate tubes were stained with the same batch of CD14-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 CD14+ 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 CD14+ Monocytes**					
CD14-Fluorescent Dye	Average (%)	Relative Difference vs. 2H				Criteria
		6H	24H	48H	72H	
cFluor V450	79.3	-4.10%	-4.18%	-0.58%	-4.44%	Relative Difference ≤10%

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 CD14-cFluor-conjugated reagent and analyzed on Cytek Northern Lights™ flow cytometer. The median of percent CD14+ monocytes at each dilution level were compared to the median of percent CD14+ monocytes at all levels, the relative difference was calculated and was within the acceptance criteria.

Specimen: CD-CHEX PLUS	**Percent CD14+ Monocytes**					
CD14-Fluorescent Dye	Relative Difference					Criteria
	Undiluted	2X dilution	4 X dilution	8 X dilution	16 X dilution	
cFluor V450	-4.68%	-1.27%	-0.00%	2.18%	2.35%	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

- Pugin J, et al. 1998. Infect Immun. 66:1174
- Wright SD, et al. 1990. Science. 249:1431