ErythroStep™ EMA

REF		
EMA-25T	25 test	RUO

1. PRODUCT DESCRIPTION

ErythoStep™ EMA is based on the selective reactivity of maleimide groups toward free sulfhydryl (—SH) residues present on proteins. Maleimide reacts specifically with accessible thiol groups under near-physiological conditions, forming a stable and irreversible thioether bond.

In intact erythrocytes, the number of exposed surface thiols is limited but detectable, primarily originating from transmembrane proteins such as band 3 (anion exchanger 1, AE1), glycophorins, and other membrane-associated proteins whose cysteine accessibility may vary depending on membrane integrity, oxidative status, and storage conditions. Upon incubation with 5-maleimido-eosin, accessible membrane thiols are covalently labeled, anchoring the eosin fluorophore to the erythrocyte membrane.

The eosin moiety is a xanthene-based fluorophore excitable at 488 nm, producing emission in the green-yellow spectrum (~540–570 nm), compatible with standard FITC/PE detection channels depending on optical configuration. Because the labeling is covalent, fluorescence remains stable and does not redistribute between cells.

Alterations in erythrocyte membrane protein composition or organization—such as those described in hereditary spherocytosis (HS)—may result in modified EMA fluorescence intensity patterns. For this reason, EMA labeling is widely used in research protocols supporting the evaluation of erythrocyte membrae disorders.

Recommended usage: ErythoStep™ EMA is a research-use-only (RUO) fluorescent thiol-reactive reagent intended for the covalent labeling of erythrocyte membrane proteins for analysis by flow cytometry. The maleimide moiety selectively reacts with accessible sulfhydryl (—SH) groups on membrane proteins, forming stable thioether bonds, while the eosin fluorophore enables bright detection upon excitation with standard 488 nm lasers.

This reagent is designed for research applications involving erythrocyte membrane characterization, redox state assessment, and membrane protein integrity studies. It may also be used in research protocols supporting the evaluation of erythrocyte membrane disorders, including studies related to hereditary spherocytosis, where alterations in membrane protein composition or accessibility may be investigated by fluorescence intensity analysis. ErythoStep™ EMA is intended for laboratory research use only and is not for use in diagnostic procedures.

Presentation: ErythroStep™ EMA is supplied as a lyophilized (desiccated) reagent in a cryovial format. Each cryovial contains sufficient reagent for 2 tests.

Storage Instruction: store ErythroStep ™ EMA at 2-8° C in dark. Do not use after expiration date stamped on vial.

Reagent provided: ErythroStep ™ EMA is supplied as a lyophilized preparation of 5-maleimido-eosin in a light-protected cryovial format. Each vial contains a pre-measured amount of reagent sufficient for two determinations under the recommended protocol. Upon reconstitution with 20 µL of sterile PBS (pH 7.2–7.4), the reagent is ready for immediate use. The formulation ensures optimal maleimide reactivity toward accessible erythrocyte membrane sulfhydryl groups under near-physiological conditions. No additional stabilizers or carrier proteins are included.

Recommendation and warnings: This product contains sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. Before acquiring samples, adjust the discriminator (threshold) to minimize debris.

Revisión: 23/02/2026

2. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer.

Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

3. PROTOCOL

A. Reagent Preparation

1. Allow the cryovial to equilibrate to room temperature before opening.
2. Add 20 µL of sterile PBS (pH 7.2–7.4) directly into the vial.
3. Gently pipette up and down to ensure complete dissolution of the lyophilized reagent.
4. Use immediately after reconstitution. Protect from light.

B. Erythrocyte Labeling Procedure

Prepare two tubes per sample:

- One labeled tube (positive tube)
- One

For each patient and each control sample, prepare two tubes.

1. Add 10 µL of whole blood to each tube.
   Add 2 mL of FACSFlow + 0.2% albumin + sodium azide.
2. Centrifuge for 5 minutes at 2000 rpm.
3. Discard the supernatant and resuspend the pellet in FACSFlow + 0.2% albumin + sodium azide.
4. Repeat wash steps (2–3) once more.
5. Transfer 2.5 µL of washed packed erythrocytes into a 1.5 mL microcentrifuge tube.
6. Add 10 µL of reconstituted ErythoStep™ EMA reagent to the positive tubes only.
   Add 10 µL of FACSFlow + 0.2% albumin + sodium azide to negative control tubes.
7. Incubate for 1 hour at room temperature, protected from light.
8. Centrifuge at maximum speed in a microcentrifuge.
9. Carefully discard the supernatant.
10. Add 1 mL of FACSFlow + 0.2% albumin + sodium azide and transfer back to cytometry tubes.
11. Centrifuge again at maximum speed.
12. Resuspend in 0.5 mL of FACSFlow + 0.2% albumin + sodium azide.
13. Transfer 100 µL to a new cytometry tube.
14. Add 1.5 mL of FACSFlow + 0.2% albumin + sodium azide.
15. Acquire a minimum of 100,000 events on a flow cytometer. Apply routine instrument calibration settings.

    Recommended FSC threshold: 200 (adjust according to instrument configuration).

4. SPECTRAL CHARACTERISTICS

ErythoStep™ EMA contains eosin, a xanthene-based fluorophore suitable for excitation with standard blue (488 nm) lasers and compatible with 532 nm excitation sources.

Spectral properties:

- **Excitation maximum:** ~488 nm (also excitable at 532 nm)
- **Emission maximum:** ~540–570 nm
- **Detection channel**: Typically compatible with FITC or PE channels depending on the optical configuration and filter set of the instrument.

Eosin provides strong fluorescence intensity and improved photostability compared to fluorescein, allowing clear discrimination of labeled erythrocytes from unlabeled populations. Because the fluorophore is covalently attached to membrane proteins through stable thioether bonds, signal stability is maintained during washing and mild fixation procedures.

Users should verify detector configuration and compensation settings according to their specific cytometer.

5. Data Analysis & Interpretation (Research Use Context – HS Support)

Erythrocytes are identified based on forward scatter (FSC) and side scatter (SSC) characteristics. Doublets and debris should be excluded using standard gating strategies.

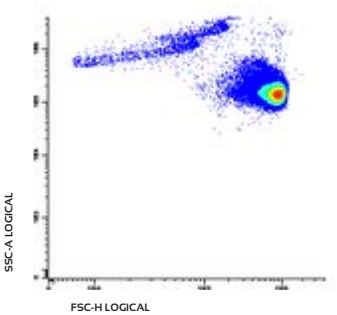

Figure 1- Dot-plot obtained using Aurora cytometer (Cytek) and Infinicyt software (BD Bioscioscienes)

Fluorescence intensity is analyzed in the appropriate detection channel (FITC/PE-equivalent depending on instrument configuration). Results are typically expressed as:

- Mean Fluorescence Intensity (MFI)
- Relative fluorescence compared to internal controls
- Fluorescence ratio between test and control samples

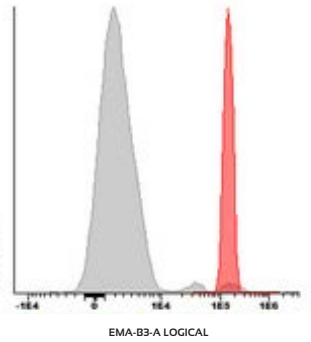

Figure 2- histogram obtained using Aurora cytometer (Cytek) and Infinicyt software (BD Bioscioscienes)

In research protocols evaluating erythrocyte membrane integrity, reduced EMA fluorescence intensity has been associated with alterations in membrane proteins, particularly band 3 (AE1), which may be quantitatively or structurally altered in hereditary spherocytosis (HS).

For studies supporting HS investigation:

- Patient samples are typically analyzed alongside healthy control samples processed under identical conditions.
- Decreased fluorescence relative to controls may reflect altered membrane protein accessibility or content.
- Interpretation should always be performed within the context of additional laboratory findings and clinical information.

ErythoStep™ EMA is not intended for standalone diagnostic use. Results are for research purposes only.

Advantages:

- **Covalent labeling:** Maleimide forms stable thioether bonds with membrane protein thiols, preventing dye transfer between cells.
- **High signal intensity**: Eosin provides bright fluorescence suitable for robust erythrocyte population discrimination.
- **Signal stability:** Compatible with washing steps and mild fixation without significant signal loss.
- **Redox sensitivity:** Capable of detecting changes in accessible thiols associated with oxidative stress or membrane alterations.
- **Standard laser compatibility:** Optimized for 488 nm excitation, available on most flow cytometers.
- **Supportive research tool for membrane disorders:** Widely used in protocols evaluating erythrocyte membrane protein abnormalities.

Limitations

- **Thiol accessibility dependent:** Labeling efficiency depends on the availability of accessible —SH groups on membrane proteins.
- **Sample handling sensitivity:** Excessive oxidation or improper storage of blood samples may affect fluorescence intensity.
- **Reductive treatments:** Strong reducing conditions may alter membrane integrity and affect results.
- **pH sensitivity:** Maleimide groups may hydrolyze under alkaline conditions (pH >7.5), reducing reactivity.
- **Photosensitive reagent:** Must be protected from light during preparation and incubation.
- **Research use only:** Not validated for diagnostic procedures.

6. REFERENCES

- King MJ, Telfer P, MacKinnon H, Langabeer L, McMahon C, Darbyshire P, Dhermy D. Using the eosin-5-maleimide binding test in the differential diagnosis of hereditary spherocytosis and hereditary pyropoikilocytosis. Cytometry B Clin Cytom. 2008 Jul;74(4):244-50. doi: 10.1002/cyto.b.20413. PMID: 18454487,

7. EXPLANATION OF SYMBOLS

	Form
	Catalog reference
	Contains sufficient for <n> test
	Quantity per test
	Regulatory Status
	Research Use Only
	Manufacturer

8. MANUFACTURED BY:

Address: Avda. Universidad de Coimbra, s/n
Cancer Research Center (C.I.C)
Campus de Unamuno
37007 Salamanca (Spain)
Telf./fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com

Revisión: 23/02/2026