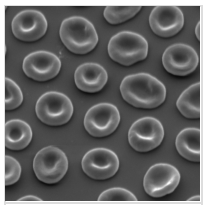

Sep 01, 2022

Osmolality-controlled fixation and simple preparation of human red blood cells for scanning electron microscopy

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dx.doi.org/10.17504/protocols.io.36wgq7xj5vk5/v1

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ABSTRACT

Scanning electron microscopy (SEM) provides a way to visualize red blood cell (RBC) morphology. Previous methods for human RBC fixation can induce osmotic-related changes to healthy RBCs, which can interfere with interpretation of biological morphological changes. In addition, traditional methods for fixation and dehydration of RBCs and associated SEM preparations involve multiple chemicals and time-intensive steps. Here, we provide a simplified protocol for human RBC fixation with careful control of osmolality. This protocol omits the use of sodium cacodylate, osmium tetroxide, and ethanol gradient dehydration steps, yet results in comparable outcomes.

DOI

dx.doi.org/10.17504/protocols.io.36wgq7xj5vk5/v1

PROTOCOL CITATION

Heather N Colvin, Glen Marrs, Regina Joice Cordy 2022. Osmolality-controlled fixation and simple preparation of human red blood cells for scanning electron microscopy. **protocols.io** https://protocols.io/view/osmolality-controlled-fixation-and-simple-preparat-ceq2tdye

FUNDERS ACKNOWLEDGEMENT

NIH
Grant ID: K01HL143112

NIGMS
Grant ID: T32 GM127261

KEYWORDS

erythrocyte, red blood cell, echinocytosis, scanning electron microscopy, osmolality, microscopy, fixation, human

CREATED

Aug 02, 2022

LAST MODIFIED

Sep 01, 2022

PROTOCOL INTEGER ID

68090

MATERIALS TEXT

PBS pH 7.4 **Thermo Fisher Scientific Catalog #10010023**

Distilled Water **Thermo Fisher Catalog #15230162**

Glutaraldehyde solution (50% in solution) **Sigma Aldrich Catalog #G6403**

Round Glass Coverslips 12 mm diameter **Thermo Fisher Scientific Catalog #50-143-822**

SAFETY WARNINGS

Human blood carries a risk of possible transmission of bloodborne pathogens. Proper personal protective equipment (PPE) in accordance with biosafety level 2 research should be used to minimize this risk.

Glutaraldehyde should be used in chemical fume hood and disposed of according to institutional guidelines.

BEFORE STARTING

In order to obtain the human blood needed for these studies, ethical clearance must first be obtained.

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1

Note the following protocol includes volumes to fix one (1) sample of packed, washed human red blood cells (RBCs). For multiple samples, scale up all volumes proportionately

Make osmolality-controlled buffer

- Add **300 µL** 1X PBS (pH 7.4) + **200 µL** distilled water (dH_2O) in microcentrifuge tube for 500uL total osmolality-controlled buffer solution
- Mix well

Osmolality ≈ 160 mmol/kg

Note: Osmolality measured with a Wescor VAPRO 5520 (vapor pressure osmometer)

2 Suspend human RBCs in osmolality-controlled buffer

Caution: Institutional bloodborne pathogen biosafety precautions should be followed when working with specimens containing unfixed human blood

- Add **25 µL** of packed, washed RBCs to **500 µL** osmolality-controlled buffer from *Step #1*
- Mix well with pipette, gently

3 Prepare osmolality-controlled 2% glutaraldehyde solution

Caution: Prepare glutaraldehyde solution in chemical fume hood

- Combine **240 µL** dH_2O, **240 µL** 1X PBS (pH 7.4), **20 µL** 50% glutaraldehyde in conical tube and mix well by inverting

Osmolality ≈ 350 mmol/kg

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Note: Osmolality measured with a Wescor VAPRO 5520 (vapor pressure osmometer)

4 Fix RBCs with osmolality-controlled glutarldehyde

30m

- Add **500 µL** osmolality-controlled 2% glutaraldehyde solution from *Step #3* slowly to well-mixed RBC suspension from *Step #2.*
- Invert gently in microcentrifuge tube
- Incubate at **Room temperature** in the dark for **00:30:00**

Final osmolality of fixing solution ≈ 260 mmol/kg

Note: Osmolality measured with a Wescor VAPRO 5520 (vapor pressure osmometer)

5 Wash glutaraldehyde-fixed RBCs

10m

- Centrifuge fixed RBCs after completion of *Step #4* at **900 x g, 00:05:00 , reduced acceleration and braking (Acc: 4/9, Dec: 2/9)**
- Remove supernatant without disturbing the RBC pellet

Properly dispose of supernatant containing glutaraldehyde per institutional guidelines

- Add **1 mL** dH_2O and mix pellet well with pipette
- Centrifuge cells again **900 x g, 00:05:00 , reduced acceleration and braking (Acc: 4/9, Dec: 2/9)**
- Remove supernatant without disturbing the RBC pellet

6 Dilute fixed, washed RBCs in dH_2O

30m

- Mix RBC pellet well with pipette and add **5 µL** of fixed RBC pellet to **995 µL** dH_2O to create at 0.5% hematocrit (hct) suspension of washed and glutaraldehyde-fixed RBCs in

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water

- Mix well and add **100 µL** 0.5% hct glutaraldehyde-fixed RBC suspension to *12mm round Poly-L-Lysine coated coverslide** on slide warmer

*Use the most appropriate slide size for the specific scanning electron microscope that will be used to image cells

- Dry slides on slide warmer for **60 °C** **Overnight**

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