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Version 1

untitled protocol V.1

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Protocol status: In development

We are still developing and optimizing this protocol

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Protocol Integer ID: 26840

Keywords: Physiology, Biology, Bioanalysis, iron concentration, erythrocyte, total iron mass, blood component, calculated total iron mass, untitled protocol cattle, sample purification, cattle, serum, purification, oleic acid

Abstract

Cattle's blood component is compressed using centrifuge (5000 rpm for 10 minutes), then supernatant (erythrocytes) collected. Erythrocytes with a ratio of 3:1 (serum:supernatant), treated with initial mixing each with HCl (0.5 M) NaOH (0.5 M), and 30% H₂O₂ with an initial ratio of 1:1 (v/v). Each variable then treated by mixing SnCl₂ (1 M, 1:1 v/v), oleic acid (2:1 v/v), and O_{2(g)} (0.5 L/m, 60s). The best sample then purified by heat about 800°C for 120 minutes. The highest calculated total iron mass is 240000 µg/100 ml (NaOH + oleic acid). Sample purification has increased the iron concentration up to 46.30% (m/m%).

Guidelines

Beware to react the blood with peroxide because it will produces bubbles

Materials

MATERIALS


 Sodium Hydroxide **Merck MilliporeSigma (Sigma-Aldrich)**

STEP MATERIALS


[M] 0.5 Molarity (M)

[M] 0.5 Molarity (M)

 37 °C

 00:30:00

 Sodium Hydroxide **Merck MilliporeSigma (Sigma-Aldrich)**

 10 rpm , Mix with sample for 20 seconds

Protocol materials

 Sodium Hydroxide **Merck MilliporeSigma (Sigma-Aldrich)**




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 Sodium Hydroxide **Merck MilliporeSigma (Sigma-Aldrich)**

Troubleshooting

Sample preparation

1

Compress the whole blood sample from cattle.  6 mL  37 °C  00:10:00

Equipment

Mini-centrifuge

NAME

Centrifuge

TYPE

Fisher

BRAND

S67601B

SKU


<https://www.fishersci.com/shop/products/fisherbrand-standard-mini-centrifuge-standard-mini-centrifuge/s67601b>

LINK

Any standard mini centrifuge with adapters for different tube sizes will suffice

SPECIFICATIONS



 5000 rpm

2

Mix  2 mL blood sample sample with  2 mL NaOH  37 °C  00:00:30

[M] 0.5 Molarity (M)

 Sodium Hydroxide Merck MilliporeSigma (Sigma-Aldrich)

Expected result

dark green solution with strong odor

🌀 10 rpm , after we rest the mixed solution for 30 seconds

- 3 Chelate reaction by adding 🧪 4 mL Oleic acid into the previous mixed sample solution then let it sit for ⌚ 00:00:30 . After that, mixed them by 🌀 10 rpm for ⌚ 00:00:30 .

Expected result

Equipment

Centrifuge

NAME

Benchtop Centrifuge

TYPE

Eppendorf

BRAND

5405000441

SKU

<https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html>

LINK

Any benchtop centrifuge will suffice

SPECIFICATIONS

