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Version 2

# New Iron Extracting Method from Cattle's Blood for Iron Concentration Analysis V.2

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Khairil Asnan<sup>1</sup>, Mohd. Sadzan Asram bin Aliming<sup>2</sup>, Aprialdy Idrus<sup>2</sup>, Diana Eka Pratiwi<sup>2</sup>

<sup>1</sup>Biology Department, Universitas Negeri Makassar; <sup>2</sup>Chemistry Department, Universitas Negeri Makassar



Khairil Asnan

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**Protocol status:** Working

We use this protocol in our group and it is working. We will develop this protocol for better result

**Created:** August 12, 2019

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**Protocol Integer ID:** 26842

**Keywords:** Physiology, Biology, Bioanalysis, Biosynthesis, Iron-extracting method, iron concentration analysis cattle, new iron extracting method, iron concentration, calculated total iron mass, total iron mass, whole blood sample, ml whole blood sample, sample purified by furnace, erythrocyte, blood component, cattle, serum

## 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 with NaOH (0.5 M) with an initial ratio 1:1 (v/v) and let it sit for 30 seconds then mixed it by centrifugation about 10 rpm for 30 seconds. Sample then treated by mixing oleic acid (2:1 v/v). The sample then dehydrated by heat about 121°C for a week. Calculated total iron mass was 240000 µg/100 ml whole blood sample (about 14.40% content of the whole sample). Sample purified by furnace using high temperature about 800°C for 2 hours and increased the iron concentration up to 46.30% (m/m%).

## Materials

### MATERIALS

 Sodium Hydroxide Merck MilliporeSigma (Sigma-Aldrich)

### STEP MATERIALS

 Sodium Hydroxide Merck MilliporeSigma (Sigma-Aldrich)

we also used oleic acid as chelating agent

## Protocol materials

 Sodium Hydroxide Merck MilliporeSigma (Sigma-Aldrich)




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## 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

