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Version 2

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

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#### **Manuscript citation:**

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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

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**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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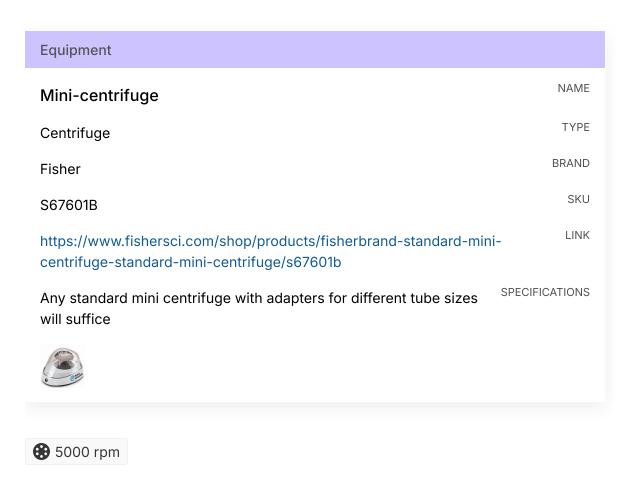
### **Troubleshooting**



### Sample preparation

1

Compress the whole blood sample from cattle. 4 6 mL 37 °C 00:10:00



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 **©** 00:00:30 .

**Expected result** 

## Equipment NAME Centrifuge TYPE **Benchtop Centrifuge BRAND Eppendorf** SKU 5405000441 LINK https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html **SPECIFICATIONS** Any benchtop centrifuge will suffice

