

Supplemental FIG S2 Summary of the preparation of the iron-depleted cation-adjusted Mueller-Hinton broth

PREPARATION OF Iron-Depleted Cation-Adjusted Mueller–Hinton Broth

1

Adding Chelex® 100



Take 1 L autoclaved MHB cooled down to room temperature.

Add 100 g Chelex® 100 chelating resin, analytical grade [100–200 mesh, Na⁺ form].

2

6-h stir + filtering

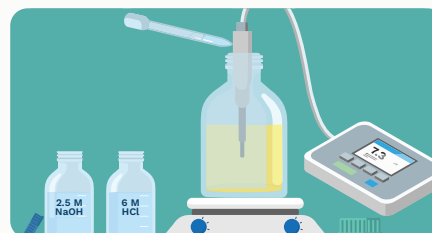


Stir at room temperature for at least 6 hours.

Filter using 0.2 µm pore-size filter to remove resin.

3

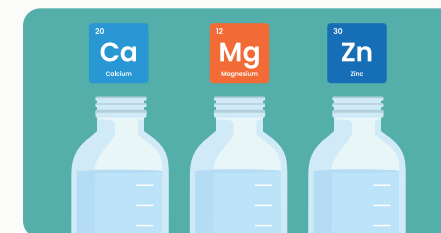
pH adjustment



Adjust pH to 7.2–7.4 by using 6 M HCl or 2.5 M NaOH.

4

Cation adjustment



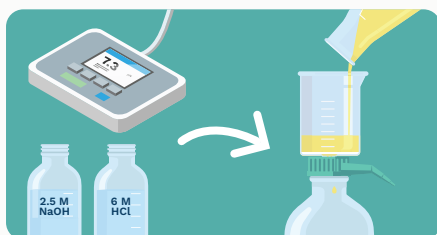
Add Ca²⁺ (final 20–25 µg/mL).

Add Mg²⁺ (final 10–12.5 µg/mL).

Add Zn²⁺ (final 0.5–1 µg/mL).

5

pH adjustment + filtering

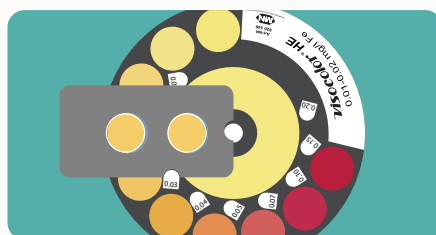


Adjust pH to 7.2–7.4 by using 6 M HCl or 2.5 M NaOH.

Filter using 0.2 µm pore-size filter.

6

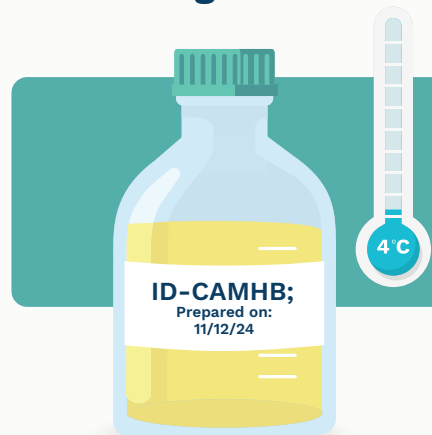
Iron concentration measurement



Measure iron concentration (final ≤0.03 µg/mL).

7

Storage at 4°C



Avoid iron contamination



To avoid contamination with iron during the preparation of the iron-depleted cation-adjusted Mueller-Hinton broth, use distilled or deionized water, plastic instead of metal spatulas, Teflon™-coated magnetic bars, and high-purity reagents.