

Supplementary Method 14 - Preparation of 0.2M EDTA pH 8.0

Reagents

- EDTA disodium salt, dihydrate
- MilliQ water
- 5M NaOH (see **Supplementary Method 13**)
- Sodium hydroxide (NaOH) pellets

Equipment

- 1L beaker
- 2L beaker
- 1L measuring cylinder
- 2L measuring cylinder
- Vacuum filter
- pH meter
- Sterile bottles
- Stirrer
- Weighing scales

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination. This buffer is made in a fume hood.

How to make 1L of 0.2M EDTA pH 8.0

1. Weigh out **74.45 g** EDTA disodium salt, dihydrate and add to a 1 L beaker.

Initials:

2. Measure out **800 mL** milliQ water and add to the beaker.

Initials:

3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the pH reaches 8.0.
4. Add a newly calibrated pH meter into the solution to observe the pH.
5. To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around **8 g** of NaOH pellets before the pH is at 8.0. (Reading.....)

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6. Fine tune pH with 5M NaOH. (Batch Number

Initials:

7. Once fully dissolved (this will take some time so be patient), transfer to 1L cylinder and top up the solution to 1L using milliQ water, if necessary.

Initials:

8. Vacuum filter into sterile bottles.
9. Label with batch and date.

Initials:

Store 0.2M EDTA pH 8.0 solution at room temperature (+15°C – +25°C).

How to make 2L of 0.2 M EDTA pH 8.0

1. Weigh out **148.89 g** EDTA disodium salt, dihydrate and add to a 2 L beaker.

Initials:

2. Measure out **1600 mL** milliQ water and add to the beaker.

Initials:

3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the pH reaches 8.0.
4. Add a newly calibrated pH meter into the solution to observe the pH.
5. To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around **16 g** of NaOH pellets before the pH is at 8.0. (Reading.....)
6. Fine tune pH with 5M NaOH. (Batch Number

Initials:

7. Once fully dissolved (this will take some time so be patient), transfer to 2L cylinder and top up the solution to 2 L using milliQ water, if necessary.

Initials:

8. Vacuum filter into sterile bottles.
9. Label with batch and date.

Initials:

Store 0.2M EDTA pH 8.0 solution at room temperature (+15°C – +25°C)

How to make 5L of 0.2 M EDTA pH 8.0

1. Weigh out **372.23 g** EDTA disodium salt, dihydrate and add to a 5 L beaker.

Initials:

2. Measure out **4000 mL** milliQ water and add to the beaker.

Initials:

3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution. The EDTA salt will not go into solution until the pH reaches 8.0.
4. Add a newly calibrated pH meter into the solution to observe the pH.
5. To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around **40 g** of NaOH pellets before the pH is at 8.0. (Reading.....)
6. Fine tune pH with 5M NaOH. (Batch Number

Initials:

7. Once fully dissolved (this will take some time so be patient), transfer to 5 L cylinder and top up the solution to 5 L using milliQ water, if necessary.

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Initials:

8. Vacuum filter into sterile bottles.
9. Label with batch and date.

Initials:

Store 0.2M EDTA pH 8.0 solution at room temperature (+15°C – +25°C).