

Reagent Preparation Worksheet

0.5 M EDTA (Disodium Dihydrate), pH 8.0 — Chelating Stock Solution

Document ID	RPW-CHM-014	Revision No.	3
Effective Date	2026-07-30	Related SOP	SOP-CHM-007
Reagent Name	0.5 M EDTA (Disodium), pH 8.0	Department	Analytical Chemistry Core
Requested By	Dr. Priya Nandakumar (Cell Biology Unit)	Work Order No.	WO-2026-0731

1 Purpose and Scope

This worksheet documents the preparation of a 0.5 M ethylenediaminetetraacetic acid (EDTA), disodium salt, stock solution at pH 8.0, together with the working dilution series required for downstream nucleic acid extraction and metal-chelation assays performed by the Cell Biology Unit. It is issued under SOP-CHM-007 (*Preparation and Standardization of Volumetric Reagents*) and constitutes the batch record for lot EDTA-0526-014. Completion of every field on this worksheet, including the prepared-by and checked-by signatures in Section 7, is required before the solutions may be released for use outside the Analytical Chemistry Core.

This worksheet is distributed to the requesting unit, retained in the Core Facility batch record binder, and archived electronically in the LIMS reagent module for the retention period defined in the Laboratory Quality Manual (7 years from expiry).

At a Glance

- Target concentration: **0.5 M**
- Batch volume: **1000 mL**
- Target pH: **8.00 ± 0.05**
- Working dilutions: **3** (10 mM, 1 mM, 0.1 mM)
- Assigned shelf life: **6 months**

Safety Classification

- NaOH pellets: corrosive (GHS05), exothermic in solution
- Required PPE: nitrile gloves, splash goggles, lab coat
- Ventilation: standard bench; fume hood not required
- SDS location: Cabinet C-12, binder Section 4

2 Reagent Identity and Source Materials

2.1 Reagent Identity

Property	Value
Chemical Name	Ethylenediaminetetraacetic acid, disodium salt dihydrate
Common Synonyms	Disodium EDTA dihydrate, EDTA-Na ₂
CAS Number	6381-92-6
Molecular Formula	C ₁₀ H ₁₄ N ₂ Na ₂ O ₈ · 2H ₂ O
Molecular Weight	372.24 g/mol
Physical Form (as received)	White crystalline powder
Target Stock Concentration	0.5 M
Target Final pH	8.00 ± 0.05 (adjusted with NaOH)
Target Batch Volume	1000 mL
Diluent	Deionized water, ASTM Type II (≥1 MΩ·cm)

2.2 Source Material, Lot, and Expiry Register

Every component lot used in this preparation must be recorded below *before* weighing begins. Lots that are expired, unlabeled, or missing a certificate of analysis must not be used; quarantine the material and notify the Core Facility supervisor.

Component	Manufacturer	Lot No.	Received	Expiry	Storage
EDTA disodium dihydrate (Cat. SC-4102)	Synapse Chemical Co.	LN26-3391	2026-02-14	2028-02-14	RT, desiccated
Sodium hydroxide pellets, ACS grade (Cat. NX-8810)	Nexa Scientific Supply	LN25-9042	2025-11-03	2027-11-03	RT, sealed
Deionized water, Type II	In-house (Meridian Biosciences)	—	2026-07-30	Use ≤24 h	Ambient, capped

Handling Precautions

Sodium hydroxide pellets are corrosive (GHS05) and dissolve exothermically. Wear nitrile gloves, splash goggles, and a buttoned lab coat. Add NaOH to the stirring solution in small increments — never add water to solid NaOH, and never add pellets faster than the solution can absorb the heat of dissolution. Work at a ventilated bench away from open flames. Refer to the Safety Data Sheet binder, Section 4 (Cabinet C-12), for full first-aid and spill guidance.

3 Molarity Calculation

The mass of solute required for a target molarity is derived from the standard relationship between moles, volume, and molecular weight:

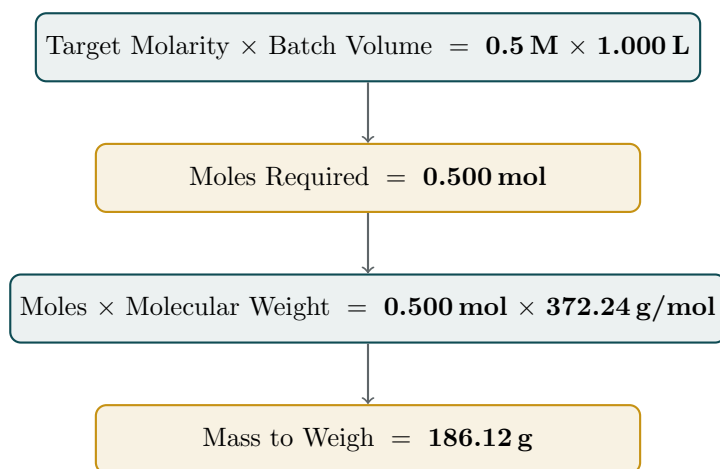
Mass-to-Weigh Calculation

$$n \text{ (mol)} = C \text{ (mol/L)} \times V \text{ (L)}$$
$$\text{mass (g)} = n \text{ (mol)} \times MW \text{ (g/mol)}$$

Worked example for this batch:

$$n = 0.5 \text{ M} \times 1.000 \text{ L} = 0.500 \text{ mol}$$
$$\text{mass} = 0.500 \text{ mol} \times 372.24 \text{ g mol}^{-1} = 186.12 \text{ g}$$

The calculation flow below traces the same four quantities from target molarity through to the mass weighed on the balance.

**💧 Dissolution and pH Adjustment Note**

Disodium EDTA dihydrate does not fully dissolve in water at neutral pH — the solution remains cloudy until the pH is raised toward 8.0. Dissolve the weighed solid in approximately 80 % of the target volume first, then raise the pH with NaOH pellets, added gradually with continuous stirring, until the solution clarifies and the pH meter reads **8.00 ± 0.05**. Typical consumption for this batch size is approximately 20 g of NaOH; treat this as a guide, not a fixed addition, and titrate to the pH endpoint rather than to a mass target.

The serial dilution scheme applied to this stock in Section 4 follows the standard $C_1V_1 = C_2V_2$ relationship for volumetric dilution of a stock solution [1].

4 Stepwise Dilution Schedule

Working concentrations are prepared from the 0.5 M stock (and from one another, in series) using the volumetric dilution relationship:

🔗 Dilution Formula

$$C_1V_1 = C_2V_2 \quad \Rightarrow \quad V_1 = \frac{C_2V_2}{C_1}$$

where C_1, V_1 describe the source (higher concentration) solution and C_2, V_2 describe the target (diluted) solution.

Step	Target	Source Solution	Source Vol.	Diluent Vol.	Mixing Method
1	10 mM in 500 mL	0.5 M EDTA stock (this worksheet)	10.0 mL	490.0 mL DI water	Stir plate, 5 min
2	1 mM in 250 mL	10 mM working solution (Step 1)	25.0 mL	225.0 mL DI water	Vortex, 30 s
3	0.1 mM in 100 mL	1 mM intermediate (Step 2)	10.0 mL	90.0 mL DI water	Vortex, 30 s

Dilution Notes

- Prepare dilutions in the order listed — each step draws from the solution made in the step immediately above it, not from the original stock.
- Use diluent (DI water) drawn from the same batch recorded in Section 2 so that all working solutions share a traceable water lot.
- Label each bottle with its concentration, preparation date, and preparer's initials *immediately* after mixing, before moving on to the next dilution, to prevent mix-ups between visually identical solutions.
- Discard any partially used working dilution bottle at the expiry assigned in Section 7; do not top up an existing bottle with fresh solution.

5 Preparation Procedure

1. Verify that the analytical balance (Asset ID BAL-014) and the benchtop pH meter (Asset ID PH-006) are within their current calibration window. If either is due within 7 days, record the concern on the Instrument Log and delay preparation until recalibration is confirmed.
2. Tare a clean, dry 250 mL beaker on the analytical balance. Weigh **186.12 g** of EDTA disodium dihydrate (Lot LN26-3391) directly into the beaker.
3. Measure 800 mL of DI water into a 1000 mL graduated cylinder and transfer it to a clean 1 L beaker fitted with a magnetic stir bar, on a stir plate at moderate speed.
4. Add the weighed EDTA to the stirring water gradually, in small portions. The solution will remain cloudy at this stage — this is expected and resolves once the pH is raised in the next step.
5. While stirring continuously, add NaOH pellets (Lot LN25-9042) in small increments, checking pH after each addition. Continue until the solution clarifies and the pH stabilizes at **8.00 ± 0.05** (approximately 20 g NaOH for this batch).
6. **Checkpoint — pH verification.** Confirm the final pH with the calibrated pH meter before proceeding. Record the reading, initials, and time below.
7. Transfer the solution to a 1000 mL volumetric flask. Rinse the preparation beaker twice with small volumes of DI water and add both rinses to the flask.
8. Bring the flask to a final volume of **1000 mL** with DI water and invert at least 10 times to mix thoroughly.
9. Filter the stock through a 0.22 μm PES bottle-top filter directly into a sterile, amber glass storage bottle.

10. Immediately prepare the three working dilutions per the schedule in Section 4, drawing from the freshly filtered stock.
11. Label the stock bottle and each working-dilution bottle per SOP-CHM-007 labeling requirements: reagent name, concentration, lot number, preparation date, expiry date, and preparer's initials.
12. Store the stock bottle at room temperature, protected from light; store all working dilutions at 4 °C.

Initials	Time	pH Reading
M.T.	14:22	8.02

6 Quality Verification

Before release, the completed stock and its working dilutions must meet the acceptance criteria below. pH measurement is performed and recorded consistent with USP General Chapter <791> [3].

Parameter	Acceptance Criterion	Method	Result	P/F
pH	8.00 ± 0.05	Calibrated benchtop pH meter (PH-006)	8.02	Pass
Appearance	Clear, colorless, free of visible particulates	Visual inspection against black/white background	Clear, colorless	Pass
Filter Integrity	Bubble point ≥ 50 psi (0.22 µm PES)	Post-use bubble point test	54 psi	Pass
Fill Volume (Stock)	1000 mL ± 5 mL	Graduated cylinder verification	998 mL	Pass

⚠ On Any Failing Result

If any parameter above fails its acceptance criterion, do not release the batch. Label the stock and all working dilutions **“DO NOT USE — QUARANTINED,”** initiate a Deviation Report per SOP-QA-002, and notify the Core Facility supervisor before any further action is taken.

The 6-month shelf life assigned to this lot (Section 7) reflects the stability data summarized in the Core Facility's internal technical bulletin on chelating-agent stock solutions [2].

7 Lot Assignment and Sign-Off

Item	Value
Assigned Lot Number	EDTA-0526-014
Preparation Date	2026-07-30
Expiry Date	2027-01-30 (6-month stability, [2])
Storage Location	Reagent Fridge R-3, Shelf 2 (working dilutions); Chemical Shelf C-12, RT (stock)
Total Stock Volume Prepared	1000 mL
Total Working Dilutions Prepared	500 mL (10 mM), 250 mL (1 mM), 100 mL (0.1 mM)

Prepared By

Name: Maria Torres

Checked By

Name: Dr. Aiden Walsh

Approved By

Name: Dr. Priya Nandakumar

Signature: _____

Signature: _____

Signature: _____

Date: _____

Date: _____

Date: _____

References

- [1] Daniel C. Harris. *Quantitative Chemical Analysis*. W. H. Freeman, 9th edition, 2015.
- [2] Meridian Biosciences, Analytical Chemistry Core Facility. Technical bulletin TB-014: Stability of chelating agent stock and working solutions under standard storage conditions. Technical Report TB-014, Meridian Biosciences, 2025.
- [3] United States Pharmacopeial Convention. General chapter <791>: ph. USP–NF General Chapters, 2023.