

0.1M EDTA-0.2M MgCl₂-0.2M Ascorbate Buffer

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Abstract

Preparation of iron chloride resuspension buffer using disodium EDTA dihydrate and magnesium chloride in Tris buffer.

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Guidelines

Recipe as developed by Seth:

Reagent (Formula Weight)	Amount	Final Concentration
Tris-base (FW=121.14)	1.51g	0.125M
Na ₂ -EDTA dihydrate (FW= 372.24)	3.72g	0.1M
MgCl ₂ hexahydrate (FW=203.3)**	4.07g	0.2M
Ascorbic Acid (FW=176.12) ¹	3.52g	0.2M
5N NaOH	~4.0ml	to pH 6.5 final
MilliQ H ₂ O	to 100ml	

¹ Oxalic acid can be substituted for ascorbic acid to improve virus infectivity. Oxalic acid dihydrate (FW=126.07), use 2.52g/100ml for 0.2M. See below for testing, but for oxalic acid buffer to stay in solution, use half the amount of MgCl₂·6H₂O (i.e., 2.035g/100ml).

**Tested recipe using 0.2M MgSO₄·7H₂O (4.93g/100ml) but still turned cloudy then white after final pH.

2X Ascorbic Acid Buffer: Keep the amount of Tris-base, water and NaOH the same, but increase the amount of EDTA, Mg and ascorbate 2x. Check the pH and add NaOH or HCl to get final pH to 6.5. If increasing 2x, you can use 1 ml for every 2 mg Fe(=1 ml for every 2L seawater precipitated).

Notes:

The original formulation for EDTA-Mg buffer used the chemical Mg-EDTA which is no longer available. The new formulation is now a sodium (Na) salt, and it only contains one Mg ion. For this reason, preparation of the resuspension buffer for iron chloride precipitates should be made from EDTA, disodium salt, and MgCl₂. The two most common forms of these chemicals is EDTA-Na₂·2H₂O (dihydrate) and MgCl₂·6H₂O (hexahydrate).

When preparing this buffer, keep in mind that EDTA needs a pH above 8.0 to dissolve, and will come out of solution when the pH drops below about 5.0. Ascorbic acid also seems to come out of solution if the pH is very high. The amount of reductant (ascorbic acid or oxalic acid) can vary between 0.125M and 0.25M; this formulation uses 0.2M. Since EDTA is dissolved first, this formulation prepares 0.125M Tris using Tris-base, which allows the EDTA to go into solution more quickly.

Recipe tested with diluted amounts of key reagents:

Reagent w/normal amount per 100ml	$\frac{1}{2}$ Na ₂ -EDTA (1.86g/100ml)	$\frac{1}{2}$ MgCl ₂ (2.04g/100ml)	$\frac{1}{2}$ Oxalic Acid·2H ₂ O (1.46g/100ml)
Tris-base 1.51g/100ml	clear; pH 10.79	clear; pH 10.82	clear; pH 10.78
Na ₂ -EDTA 3.72g/100ml	clear	clear	clear
MgCl ₂ ·6H ₂ O 4.07g/100ml	clear; pH 7.68	clear; pH 4.89	clear; pH 4.59
5N NaOH	none; pH 7.68	1.25ml; pH 7.23	1.5ml; pH 7.51
Oxalic acid·2H ₂ O 2.52g/100ml	white; pH 1.68	cloudy; pH 3.02	white; pH 3.30
5N NaOH	6.75ml; cleared ~pH 4.5; but turned white at pH 6.5; total 5N NaOH=6.75ml	6.25ml; cleared ~pH 4.5; but turned a little cloudy at pH 6.5; total 5N NaOH=7.5ml	3.75ml; never cleared; became cloudy then white at pH 6.5; total 5N NaOH=5.25ml
QS to 100ml with H ₂ O	pH 6.59; white	pH 6.58; cloudy	pH 6.61; white
Final results	worst of all after 2hr	looks best after 2hr	intermediate after 2hr

Photo of solutions after final pH:



Note the $\frac{1}{2}$ MgCl₂ beaker on the left is the most clear but still a little cloudy. The $\frac{1}{2}$ Oxalic acid is very cloudy, but can still see the stir bar at the bottom. The $\frac{1}{2}$ EDTA has an obvious white precipitate at the bottom that will not go back into solution. This picture is about 2 hr after the final pH and QS to 100ml. Stirring does not make the cloudiness or precipitates go into solution.

Protocol

1x Buffer

Step 1.

Dissolve 1.51g Tris-base in 80ml Milli Q water.

1x Buffer

Step 2.

Dissolve 3.72g Na₂-EDTA dihydrate into solution.

⊕ NOTES

Bonnie Poulos 15 Jun 2015

pH will be ~10.0

1x Buffer

Step 3.

Once EDTA is in solution, dissolve 4.07g MgCl₂.

⊕ NOTES

Bonnie Poulos 15 Jun 2015

pH will drop to ~8.0

1x Buffer

Step 4.

Add 3ml of NaOH.

⊕ NOTES

Bonnie Poulos 15 Jun 2015

This will drop the pH to ~4.5 and the solution will become cloudy which indicates that the EDTA is coming out of solution.

1x Buffer

Step 5.

Dissolve the reductant (3.52g of ascorbic acid or 2.52g of oxalic acid).

⊕ NOTES

Bonnie Poulos 15 Jun 2015

The pH will increase to ~8.3 and the solution will clear up.

■ ANNOTATIONS

Uri Neri 10 Apr 2018

Dear Bonnie,

We're trying to create the resuspension buffer for the VLPs-Iron precipitates resuspension step, with the reductant agent being oxalic acid (anhydrous, FW=90.04). net weight for the acid concentration was calculated to adjust for the anhydrosity and is ~1.808 [g]. Other than this minor change, I've made no modifications to the protocol, yet I am unable to create the buffer without the solution becoming 'murky' after the addition of 3ml NaOH(5N), and after some time without stirring, visible precipitates are formed (and the solution becomes clear).

We've followed the protocol to the letter, and continuously measured pH.

Your thoughts on the matter would be greatly appreciated.

With best regards,

Uri Neri

1x Buffer

Step 6.

Once the reductant is in solution, add the last 1ml of NaOH.

1x Buffer

Step 7.

Check the pH using pH paper (the buffer should be at pH 6.0 - 6.5)

📌 NOTES

Bonnie Poulos 23 Jun 2015

The solution may need some minor adjusting with NaOH or HCl to achieve a pH of 6.0.

Bonnie Poulos 23 Jun 2015

pH 6.0 is ideal for good recovery of viruses.

1x Buffer

Step 8.

Check the volume and add MilliQ water for a total volume of 100ml.

1x Buffer

Step 9.

Store the buffer in the dark (bottle wrapped in foil) and visually inspect prior to use. It should be clear without precipitates.

📌 NOTES

Bonnie Poulos 15 Jun 2015

At this point, 10-15ml of buffer can be sacrificed for a final pH check using a pH meter.

Bonnie Poulos 23 Jun 2015

The buffer will start to change color after about 24 hours. It is okay to use if slightly discolored, but do not use after about 36 hours (eventually the buffer will turn almost orange!).

Warnings

When preparing this buffer, keep in mind that EDTA needs a pH above 8.0 to dissolve, and will come out of solution when the pH drops below about 5.0. Ascorbic acid also seems to come out of solution if the pH is very high. The amount of reductant (ascorbic acid or oxalic acid) can vary between 0.125M and 0.25M; this formulation uses 0.2M. Since EDTA is dissolved first, this formulation prepares 0.125M Tris using Tris-base, which allows the EDTA to go into solution more quickly.