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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.

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_2O	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 $MgCl_2$-$6H_2O$ (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 MgCl26H2O (hexahydrate).

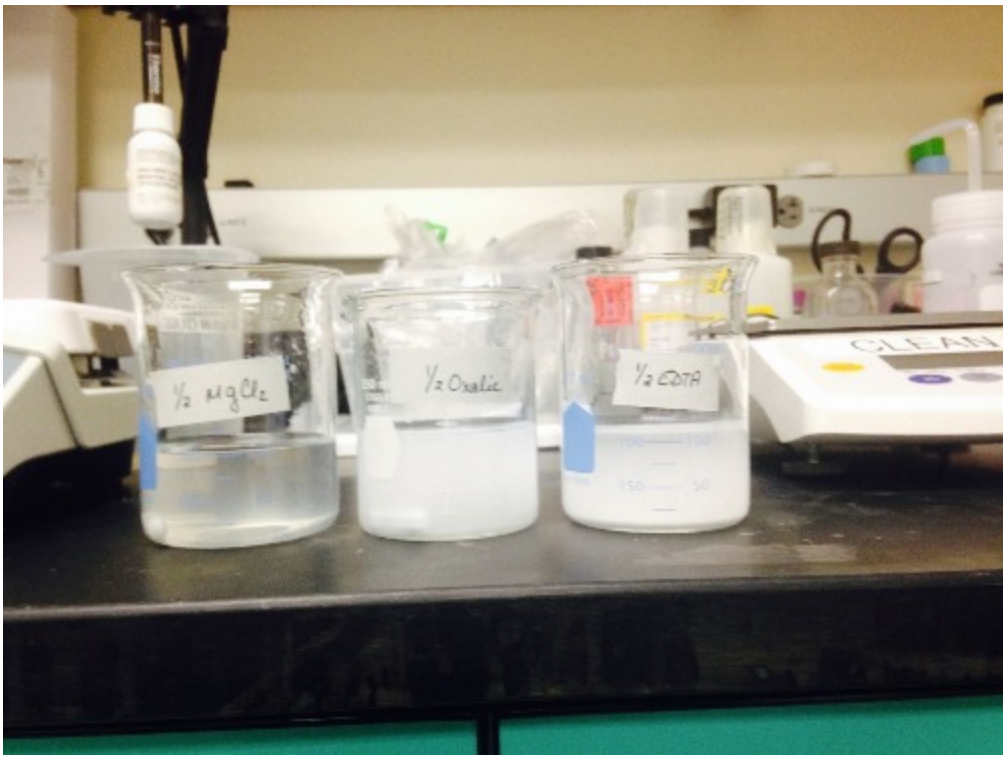
When preparing this buffer, keep in mind that EDTA needs a pH above 8.0 to dissolve, and will come out of solution when the pH drops below about 5.0. Ascorbic acid also seems to come out of solution if the pH is very high. The amount of reductant (ascorbic acid or oxalic acid) can vary between 0.125M 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	½ Na_2-EDTA (1.86g/100ml)	½ $MgCl_2$ (2.04g/100ml)	½ Oxalic Acid·$2H_2O$ (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

	$MgCl_2 \cdot 6H_2O$ 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·$2H_2O$ 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_2O	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 ½ $MgCl_2$ beaker on the left is the most clear but still a little cloudy. The ½ Oxalic acid is very cloudy, but can still see the stir bar at the bottom. The ½ 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.

Troubleshooting

Safety 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.

1x Buffer

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

2 Dissolve 3.72g Na2-EDTA dihydrate into solution.

Note

pH will be ~10.0

3 Once EDTA is in solution, dissolve 4.07g MgCl2.

Note

pH will drop to ~8.0

4 Add 3ml of NaOH.

Note

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

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

Note

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

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

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

Note

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

Note

pH 6.0 is ideal for good recovery of viruses.

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

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

Note

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

Note

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!).