The University of Iowa

At the Forefront of
Free Radical Research

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DETAPAC (DTPA), aqueous stock solution

(1988, updated 2007-04-19; 2008-01-02; 2008.07.01)

1. Introduction/Background

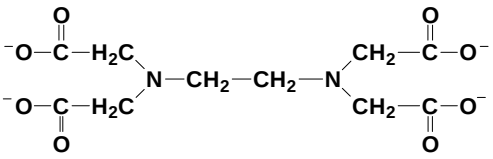
Diethylenetriaminepentaacetic acid (DTPA or DETAPAC) is a metal chelating agent that was introduced to the free radical community in 1978 as a tool to modulate the catalytic activity redox active metals in superoxide-generating systems [1,2,]. It was later demonstrated that it is also effective in blunting the catalysis of ascorbate oxidation by both iron and copper [3,4,5,]. DETAPAC is a cousin of EDTA (See structures below.), but the Fe(III)DETAPAC complex is much more slowly reduced by superoxide (and ascorbate) than Fe(III)EDTA [6], **Table 1**.

$$O_2^{\bullet -} + Fe^{3+}L_n \longrightarrow Fe^{2+}L_n + O_2 \qquad (1)$$

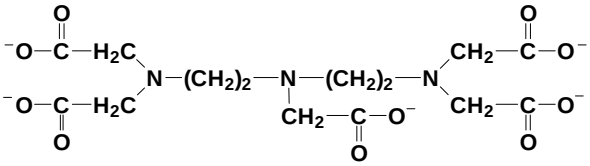
Table 1. Rate Constants for Reaction 1 [7,8,9]

Ligand	k_1/M^{-1} s^{-1} (pH 7.4)
EDTA	2×10^6
DETAPAC	$< 10^4$
HEDTA	7.6×10^5
Desferal	10^2-10^3
Transferrin	$\leq 10^5$

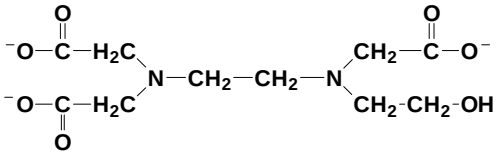
DETAPAC (DTPA) is shown in the fully deprotonatated form. However, it will not be fully deprotonated at near neutral pH values. The ususal suppliers sell DTPA as the penta-acid. As such it is very difficult to dissolve and produces a very acidic solution. For most biochemical and biological experiments we add some NaOH to the stock solution to partially neutralize the DTPA, producing a near neutral solution that is much more easily dissolved and will not challenge the pH of the solution to which it is being added.



EDTA Ethylenediaminetetraacetate anion



DETAPAC Diethylenetriaminepentaacetate anion



HEDTA Hydroxyethylethylenediaminetriacetate anion

2. Materials

Source: Example, Aldrich 09,390-2 Sigma D-1133 (1 kg)

FW = 393.35 Da for the pentaacid, $C_{14}H_{23}N_3O_{10}$ #CAS 67-43-6

The acid dissociation constants for DTPA are [10, 11]:

H_5A $pK_1 = 1.79$
$pK_2 = 2.56$
$pK_3 = 4.42$
$pK_4 = 8.76$
$pK_5 = 10.42$

3. Procedure:

<u>To make 50 mL of 10 mM stock solution of DETAPAC:</u>

$$\frac{393.35\ g}{mole} \times \frac{0.01\ mole}{1\ L} \times 0.05\ L \times \frac{1000\ mg}{1\ g} = 196.67\ mg$$

<u>How much NaOH should we add to dissolve the DTPA easily and get a near-neutral solution?</u>

Looking at the pK_as it would appear we should add about 3.4 equivalents of base per mole of DETAPAC. In this stock solution (0.01 M, 50 mL) we have a total of 0.5 mmole of DETPAC. Thus, we should add:

0.5 mmol x 3.4 = 17 mmol of NaOH; this much NaOH would be contained in 1.7 mL of 1 M NaOH, or 17 mL of 0.1 M NaOH.

Thus, to make 50 mL of a 0.01 M solution of DETAPAC

1. To a 50 mL volumetric flask add 1.7 mL of 1.0 M NaOH solution (17 mL of 0.1 M);
2. add 197 mg of DETAPAC, pentaacid form;
3. add clean water (DI, Nanopure, Milli-Q, whatever) for a total volume of approximately 40-45 mL;
4. dissolve by stirring or sonication, we prefer sonication;
5. adjust pH to approximately 7, or 7.4 or whatever is best for most proposed uses;
6. dilute to mark.

Scale up or down, of course, for other volumes or concentrations for stock solutions. DETAPAC is typically used at 0.1 mM or less in experiments, so a 10 mM stock is convenient.

4. Hazards: The bottle of solid DTPA and MSDS for DTPA have many hazardardous warnings. However, EDTA comes with these same warnings. We eat EDTA and DTPA is given *i.v.* to remove heavy metals, *e.g.* plutonium. Thus, prudent handling is in order, but perspective should be maintained.

5. References:

1 Buettner GR, Oberley LW, Leuthauser SWHC. (1978) The effect of iron on the distribution of superoxide and hydroxyl radicals as seen by spin trapping and on the superoxide dismutase assay." *Photochem Photobiol* **28:** 693-695. http://www.healthcare.uiowa.edu/CoreFacilities/esr/publications/buettnerpubs/pdf/P&P-1978-28-693-GRB-Fe-SOD.pdf

2 Buettner GR, Oberley LW. (1978) Considerations in the spin trapping of superoxide and hydroxyl radicals in aqueous systems using 5,5-dimethyl-1-pyrroline-1-oxide. *Biochem Biophys Res Commun* **83:** 69-74. [http://www.healthcare.uiowa.edu/CoreFacilities/esr/contact/buettner/pdf/BBRC-1978-83-69-DMPO.pdf] or [http://dx.doi.org/10.1016/0006-291X(78)90398-4]

3 Buettner GR. (1986) Ascorbate autoxidation in the presence of iron and copper chelates. *Free Rad Res Commun* **1:** 349-353. http://www.healthcare.uiowa.edu/CoreFacilities/esr/publications/buettnerpubs/pdf/FRR-1986-1-349-grb-AscH-metals.pdf

4 Buettner GR. (1988) In the absence of catalytic metals, ascorbate does not autoxidize at pH 7: Ascorbate as a test for catalytic metals. *J Biochem Biophys Meth* **16:** 20-40. http://www.healthcare.uiowa.edu/corefacilities/esr/publications/buettnerpubs/pdf/JBBM-1988-16-27-GRB-Ascorbate_test.pdf or [http://dx.doi.org/10.1016/0165-022X(88)90100-5]

5 Buettner GR, Jurkiewicz BA. (1996) Catalytic metals, ascorbate, and free radicals: combinations to avoid. *Radiation Research* **145:**532-541. http://www.healthcare.uiowa.edu/corefacilities/esr/publications/buettnerpubs/pdf/RadRes-1996-145-532-Buettner-Acs.pdf or http://www.jstor.org/stable/3579271

6 Buettner GR, Doherty TP, Patterson LK. (1983) The kinetics of the reaction of superoxide with Fe(III)EDTA, Fe(III)DETAPAC and Fe(III)HEDTA. *FEBS Lett* **183:** 143-146. [http://www.healthcare.uiowa.edu/corefacilities/esr/publications/buettnerpubs/pdf/13FEBS-1983-158-143-FeDETAPAC.pdf] or [http://dx.doi.org/10.1016/0014-5793(83)80695-4]

7 Ross A.B., Mallard W.G., Helman W.P., Buxton G.V., Huie R.E., Neta P. (1994) NDRL-NIST Solution Kinetics Database: Ver. 2.0. National Institute of Standards and Technology, Gaithersburg. The modern version can be accessed via the web at; http://www.rad.nd.edu/rcdc/index.html

8 Buettner G.R., Doherty T.P., Patterson L.K. (1983) The kinetics of the reaction of superoxide with Fe(III)DETAPAC and Fe(III)HEDTA. *FEBS Lett*. **183**:143-146. [http://www.healthcare.uiowa.edu/corefacilities/esr/publications/buettnerpubs/pdf/13FEBS-1983-158-143-FeDETAPAC.pdf] or [http://dx.doi.org/10.1016/0014-5793(83)80695-4]

9 Buettner, G.R. (1987) Activation of oxygen by metal complexes and its relevance to autoxidative processes in living systems. *Bioelectrochem. Bioenergetics*, **18**: 29-36. [http://www.healthcare.uiowa.edu/corefacilities/esr/publications/buettnerpubs/pdf//BioelectrochemBioen-1987-18-29-grb-Metals.pdf] or [http://dx.doi.org/doi:10.1016/0302-4598(87)85005-5]

10 Sille´n LG, Martell AE. (1964) *Stability Constants of Metal-Ion Complexes, Special Publication No. 17*; The Chemical Society: London.

11 Sille´n LG, Martell AE. (1971) *Stability Constants of Metal-Ion Complexes Supplement No. 1*, *Special Publicaton No. 25*; The Chemical Society: London.