

TRYPsinIZATION REAGENTS

1. Tris Buffer - 2M Tris HCl pH 7.4

10 ml Tris 4M $\left(\frac{4.84\text{ gm}}{10\text{ ml}}\right)$ (4M Tris = $\frac{4 \times 121.1\text{ gm}}{1000\text{ ml}}$)

8 ml HCl 4M (4M HCl = 3.33 ml HCl conc. diluted to 10 ml in distilled H₂O)

18 ml titrate to pH 7.4 with 4M HCl and q.s. to 20 ml.

Millipore filter to sterilize. Refrigerate. (Tris will not freeze.)

2. Versene Solution

8 gm NaCl

.4 gm KCl

.2 gm Versene EDTA

.005 gm Phenol Red (or 1.0 ml GIBCO stock .5%)

10 cc Tris Buffer 2M, pH 7.4

1000 cc

[If making versene to make frozen trypsin, delete 10 cc/L Tris Buffer.]

Millipore Filter. Keep at room temperature.

3. Trypsin

.05% = .50 mg recrystallized Trypsin/99 ml versene without Tris. Freeze in small aliquots. Add 1 ml Tris/100 ml solution when using frozen samples.

.25% trypsin = 250 mg trypsin/100 ml versene

Trypsin is acidic, if used in higher concentration than .25%, pH of Versene must be 7.6 so that final pH of Trypsin solution will be 7.4