

Supplementary Method 10 Preparation of TET Buffer

Reagents

- RNase free -Sigma 270733
- 0.5 M EDTA, pH 8.0
- 1 M Tris-HCl, pH 8.0
- Tween 20

Equipment

- 2L single use cell culture bottle
- 5L single use cell culture bottle
- Vacuum filter
- sterile bottles

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination. This buffer is made in a tissue culture hood to reduce the possibility of contamination by RNAses.

How to make 2 L TET

1. Add **1800 mL** Water (RNase free -Sigma 270733) to a 2L single use cell culture bottle.

Initials:

2. Add **4 mL** 0.5 M EDTA, pH 8.0.

Initials:

3. Add **20 mL** 1 M Tris-HCl, pH 8.0

Initials:

4. Add **1 mL** Tween-20

Initials:

5. Make up to 2000 mL Water (RNase free -Sigma 270733). Swirl very well to ensure that Tween-20 is well mixed in.

Initials:

6. Vacuum filter into sterile bottles.
7. Label with batch number and date.

Initials:

Store TET buffer solution at room temperature (+15°C – +25°C).

Supplementary Method 10 Preparation of TET Buffer

How to make 5L TET

1. Add **4500 mL** Water (RNAse free -Sigma 270733) to a 5L single use cell culture bottle.

Initials:

2. Add **10 mL** 0.5 M EDTA, pH 8.0.

Initials:

3. Add **50 mL** 1 M Tris-HCl, pH 8.0

Initials:

4. Add **2.5 mL** Tween-20

Initials:

5. Make up to 5000 mL Water (RNAse free -Sigma 270733). Swirl very well to ensure that Tween-20 is well mixed in.

Initials:

6. Vacuum filter into sterile bottles.
7. Label with batch number and date.

Initials:

Store TET buffer solution at room temperature (+15°C – +25°C).