Supplementary Method 11 - Preparation of 5M L6 guanidinium thiocyanate virus inactivation buffer

Reagents

- Guanidinium thiocyanate (GuSCN)
- 0.1M Tris HCl (see **Supplementary Method 12**)
- 0.2M EDTA pH 8.0 (see **Supplementary Methods 13 and 14**)
- Triton X-100

Equipment

- 1L/ 2L/ 3L/ 5L beaker
- Vacuum filter
- 2ml Screw cap tubes (Sarstedt)
- Heater/Stirrer
- Sterile bottles
- Weighing scales

Procedure

NOTE: Masks should be worn when making this buffer if there is a possibility of asymptomatic COVID-19 infection causing contamination.

Upon contact with acids, GuSCN can produce a toxic gas (HCN). As a precaution, this buffer is prepared in a fume hood.

How to make 1L L6 5M Guanidinium thiocyanate Inactivation buffer

1. Weigh out **600 g** GuSCN and add to a 1 L beaker

Initials:

2. Measure out **500 mL** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to beaker.

Initials:

3. Measure out **110 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) pH 8.0 and add to beaker

Initials:

4. Add **13 mL** Triton X-100 to beaker and stir well and heat to 60°C if required.
5. Vacuum filter into sterile bottles.
6. Label with batch and date.

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15°C – +25°C).

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How to make 2L L6 5M Guanidinium thiocyanate Inactivation buffer

1. Weigh out **1200 g** GuSCN and add to a 2 L beaker

Initials:

2. Measure out **1000 mL** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to beaker.

Initials:

3. Measure out **220 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) and add to beaker

Initials:

4. Add **26 mL** Triton X-100 to beaker and heat to 60^{o}C if required.
5. Vacuum filter into sterile bottles.
6. Label with batch and date.

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15^{o}C – +25^{o}C).

How to make 4L L6 5M Guanidinium thiocyanate Inactivation buffer (Note this method makes about 4.3 L)

1. Measure out **1.6 L** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to 5L beaker.

Initials:

2. Weigh out **2.4 Kg GuSCN** in aliquots using a 3L beaker and carefully add to 5L beaker whilst stirring and heating to 60 deg C. Rinse 3L beaker with **0.4 L** 0.1 M Tris HCl. Stir manually with sterile 10 ml pipette if required.

Initials:

3. Measure out **440 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) and add to beaker.

Initials:

4. Add **52 mL** Triton X-100 to beaker. Swirl/stir well to mix.

Initials:

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

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15^{o}C – +25^{o}C).

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How to make 5L L6 5M Guanidine thiocyanate Inactivation buffer (Note this method makes about 5.4 L)

1. Measure out **2.0 L** 0.1 M Tris HCl (see **Supplementary Method 13**) and add to 5L beaker out

Initials:

2. Weigh out **3 Kg GuSCN** in aliquots using a 3L beaker and carefully add to 5L beaker whilst stirring and heating to 60 deg C. Rinse 3L beaker with **0.5L** 0.1 M Tris HCl. Stir manually with sterile 10 ml pipette if required.

Initials:

3. Transfer to 5 L single use tissue culture bottle. Measure out **550 mL** 0.2 M EDTA pH 8.0 (see **Supplementary Method 15**) and add to bottle

Initials:

4. Add **65 mL** Triton X-100 to bottle. Swirl/stir well to mix.

Initials:

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

Initials

Store L6 inactivation buffer in dark at solution at room temperature (+15^{o}C – +25^{o}C).

Following preparation of L6 5M Guanidine thiocyanate Inactivation buffer:

1. Within a tissue culture hood cupboard, aliquot 1 ml of L6 5M guanidine thiocyanate buffer into 2ml tubes and replace lids (**see Appendix**)

2. Label with batch and date and store in dark at RT

3. After the aliquoting is complete, change gloves.

4. Place tubes in a box clearly marked 5M guanidine thiocyanate L6 Lysis buffer.

5. Clean the hood, dispose of waste and switch off tissue culture hood.

Note:5M Guanidine thiocyanate is stable at room temperature for at least three weeks. (Boom et al, J Clin Microbio 1990. 28, 3, 485-503. Commercial stocks have shelf life of 18 months)

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Appendix:

Racking tubes prior to aliquoting 5M L6 guanidinium thiocyanate virus inactivation buffer

Equipment

- 2ml screw cap tubes Sarstedt 72.694.005 or similar
- Racks (96 position)
- Empty 200 µl Tip Boxes

Procedure

NOTE: Masks should be worn when performing this procedure due to the possibility of asymptomatic COVID-19 infection causing contamination.

Lab coat, gloves, safety glasses and face mask (or face shield) must be worn. A new lab coat should be used each day. Please put your name on your lab coat. Safety glasses should not be shared but be labelled and kept for each person.

Please observe social distancing of 2 m and avoid other areas of the lab where making of buffers could be in process.

1) Make sure work area is clean and clear.
2) Unscrew lid. Place lid in box and tube in rack. Continue until 93 tubes are in rack with two spaces left.
3) Once complete, wrap filled rack in foil and place in transparent really useful box.
4) Continue as required then make sure area is left clean and tidy. Empty tube bags should go in green recycling bin. Leave cardboard boxes for disposal in consolidation space. Lab coats should be placed in the laundry bags, masks in yellow burn bin and gloves in yellow tiger stripe bin.