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# Preparation of 5M Guanidine thiocyanate L6 Inactivation Buffer

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bfevjje6

Crick COVID-19 Consortium

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ABSTRACT

This protocol is part of the Crick COVID-19 RT-PCR Testing Pipeline collection.

**THIS DOCUMENT MUST BE READ AND UNDERSTOOD BY STAFF USING IT AND DOCUMENTED EVIDENCE PROVIDED THEREOF.**

## Purpose of examination / Clinical relevance

At the end of 2019, several pneumonia cases were reported in Wuhan, China and the pathogen was confirmed as a new viral strain. World Health organization has named the newly identified coronavirus as 2019-nCoV, also known as COVID19. The disease developed into a dangerous pandemic, posing major challenges to the NHS. Although more research is necessary to better understand the virus, in response to the emergency, simple and rapid testing is essential to identify the virus in infected individuals. This will aid the implementation of efficient interventions to contain the spread, and distinguish healthcare workers who have been infected, and are required to self-isolate, from those showing similar symptoms but which are not 2019-nCoV associated. The latter category may continue to work, alleviating stress on hard-pressed healthcare resources. 2019-nCoV is an RNA virus, and the diagnostic tests detect viral RNA in swabs from patient airways using a reverse transcriptase PCR assay. Samples are submitted to HSL, an accredited reporting laboratory, and transferred to the Crick for testing.

## Principles of Examination

This procedure involves the preparation of L6 5M Guanidine thiocyanate virus inactivation buffer for the viral inactivation protocol.

GUIDELINES

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## Grade of operator

All qualified members of staff who have been signed off as competent and supervised trainees.

## Disposal

If the need arises to dispose of tubes or plates (e.g. due to spillage), all contaminated tubes and reagents used are discarded into plastic disposable jars and then into autoclave waste bins. All sharps are placed in a sharps container prior to incineration.



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MATERIALS TEXT

**Reagents**

- Guanidine thiocyanate
- 0.1 M Tris HCl, pH 6.4
- 0.2 M EDTA, pH 8.0
- Triton X-100

**Equipment**

- 1 L / 2 L / 3 L / 5 L beaker
- Vacuum filter
- 2 ml screw cap tubes sarstedt
- Heater/Stirrer
- Sterile bottles
- 5 L single use tissue culture bottle
- Weighing scales

SAFETY WARNINGS

**Upon contact with acids, GuSCN can produce a toxic gas (HCN). As a precaution, GuSCN-containing buffers are prepared in a fume hood.**

### Health and Safety

All practices must be carried out in accordance with the current health and safety policies and procedures. If in any doubt about the aspects of health and safety concerning this procedure, seek advice from the departmental Safety Officer or the health and safety team. This procedure should be carried out in a fume hood. For hazards, risks and appropriate control measures identified in the risk assessment relevant to this procedure.

### PPE

General personal protective equipment (PPE) Control Measures for laboratory work include the wearing of closed toe footwear, laboratory coat, appropriate disposable gloves (nitrile for general work or specified gloves for chemical work), and safety spectacles should be worn throughout this procedure.

### Spillage

The spill kits provided for use in the department can be used for both biohazard and chemical spills. If a spill does occur follow the procedure within the spill kit.

## Preparation of L6 Lysis Buffer

1 Please select between the recipes for *1 L, 2 L, 4.3 L* and *5.4 L L6 5 M Guanidine thiocyanate Inactivation buffer*.

**Upon contact with acids, GuSCN can produce a toxic gas (HCN). As a precaution, GuSCN-containing buffers are prepared in a fume hood.**

Step 1 includes a Step case.

**1 L L6 Buffer**
**2 L L6 Buffer**
**4.3 L L6 Buffer**
**5.4 L L6 Buffer**

step case

### 1 L L6 Buffer



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

3 Measure out **500 ml 0.1 M Tris HCl** and add to beaker.

4 Measure out **110 ml 0.2 M EDTA** , pH **8.0** and add to beaker. Stir and heat to **60 °C** if required.

5 Once GuSCN is dissolved, add **13 ml Triton X-100** to beaker and stir well.

6 Vacuum filter into sterile bottles.

7 Label with batch and date.

8 Store L6 inactivation buffer in dark at solution at **Room temperature** ( **15 °C** – **25 °C** ).

Following Preparation of L6 Lysis Buffer

9 Within a tissue culture hood cupboard, aliquot **1 ml 5 M guanidine thiocyanate** into 2 ml tubes and replace lids.

10 Label with batch and date and store in dark at **Room temperature** .

11 After the aliquoting is complete, change gloves.

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

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

5 M Guanidine thiocyanate is **stable** at **Room temperature** for at least **three weeks**.

(Boom et al, J Clin Microbiol 1990. 28, 3, 485-503 . Commercial stocks have shelf life of 18 months)



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