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# Preparation of TET Buffer

In 1 collection

Nicola O'Reilly[1], Svend Kjaer[1], Maria Greco[1]

[1]The Francis Crick Institute

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

Crick COVID-19 Consortium

Jerome Nicod
The Francis Crick Institute

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 TET buffer for the RNA extraction 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

MATERIALS



**Citation:** Nicola O'Reilly, Svend Kjaer, Maria Greco (05/16/2020). Preparation of TET Buffer. https://dx.doi.org/10.17504/protocols.io.bfeujjew

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Water for HPLC | 270733 | Sigma Aldrich |

MATERIALS TEXT

**Reagents**

- RNase free Water - (Sigma, cat no. 270733)
- 0.5 M EDTA, pH 8.0
- 1 M Tris-HCl, pH 8.0
- Tween-20

**Equipment**

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

SAFETY WARNINGS

**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.

1 Please select between the recipes for *2 L TET* and *5 L TET*.
Step 1 includes a Step case.
**2 L TET**
**5 L TET**

step case

## 2 L TET

2 Add **1800 ml RNase free Water** to a 2 L single use cell culture bottle.

3 Add **4 ml 0.5 M EDTA** , pH **8.0** .

4 Add **20 ml 1 M Tris-HCl** , pH **8.0** .

5 Add **1 ml Tween-20** .



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6 Make **up to 2000 ml Water** (RNAse free). *Swirl very well* to ensure that Tween-20 is well mixed in.

7 Vacuum filter into sterile bottles.

8 Label with batch number and date.

9 Store TET buffer at solution at **Room temperature** ( **15 °C** – **25 °C** ).



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