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extraccion de ARN en TRIzol

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ABSTRACT

Protocolo para extraccion de ARN a partir de TRIzol para virus de la fiebre aftosa

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KEYWORDS

RNA, purification, extraction

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Preparación

1 Controlar cada uno de los siguiente puntos (cada volumen es para 1 muestra a procesar)

1.1 Enfriar la centrifuga refrigerada.

1.2 Limpiar mesada de trabajo.

1.3 200 µL de cloroformo.

1.4 1 µL de glucógeno.



1.5 700 µL de isopropanol.

1.6 1 mL de Etanol 70% Agua DEPC 30%

1.7 12 µL de Agua Libre de Nucleasas

Metodo

3h 3m 15s

2 Asegurarse que las muestras congeladas con TRIZOL estén totalmente descongeladas o agregar 200 µL de muestra^{5m} a 1 mL de TRIZOL. Incubar a T. Amb  **Room temperature** . Por 5 min.  **00:05:00**

3 Agregar  **200 µl** de Cloroformo al tubo.

4 Mezclar con vortex o por inversión por  **00:00:15** Dejar a  **Room temperature** por  **00:03:00**

3m 15s

5  **13000 rpm, 2-8°C** por  **00:15:00** .

15m

- 6 Rotular un eppendorf de 1,5 mL y agregar  **1 µl** de glycogen.
- 7 Tomar la mayor cantidad posible de la fase líquida superior ( **600-700 µl**) de los tubos centrifugados y agregar al tubo correspondiente con glycogen.
- 8 Agregar un volumen equivalente de Isopropanol  **-700 µl** a cada tubo.
- 9 Mezclar con vortex unos segundos.
- 10 Incubar a  **Room temperature** por  **00:10:00** , 10m
- 11 Incubar (alternativamente)  **01:00:00** a  **-70 °C** u  **Overnight** a  **-20 °C** . 2h
- 12 Centrifugar por  **00:15:00** a  **13000 rpm** a  **2-8 °C** . 15m
- 13 Descartar sobrenadante y agregar  **1 mL** de etanol 70% Agua DEPC 30% a cada tubo. Mezclar suavemente con los dedos.
- 14 Centrifugar por  **00:15:00** A  **13000 rpm** a  **2-8 °C** (sacar fuera del freezer todos los reactivos ARN y prender el baño de agua a  **70 °C**). 15m
- 15 Remover todo el sobrenadante posible y dejar secar al aire.
- 16 Resuspender el pellet de ARN agregando  **15 µl** de Agua DEPC.
- 17 Conservar en hielo ( **On ice**) para proseguir con la RT o congelar a  **-70 °C** .