

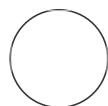


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Retina and RPE/Choroid RNA Extraction Protocol

Chottiwatt
Jittprasong¹

¹City University of Hong Kong



Chottiwatt Jittprasong

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ABSTRACT

This protocol involves the extraction of RNA from retinal and retinal pigment epithelium (RPE) cells in the subject's retina, utilizing TRIzol as the main reagent for cell lysis.

SAFETY WARNINGS




TRIzol is extremely corrosive and toxic. Exercise extreme caution while handling TRIzol (e.g. handling within the fume hood).


BEFORE START INSTRUCTIONS

Prior to the initiation of this protocol, extraction of the retinal sample from the subject's eyeball is required.

RNA Extraction

1h 48m

1 Precool the centrifuge to  4 °C

2 Add  1000 µL of **TRizol** per retina and pipette up and down 30 times to homogenize the mixture.



Safety information

This step should be done in fume hood due to toxicity of TRizol.

3 Incubate for  00:05:00  On ice



5m


4 Add  200 µL of **Chloroform** per  1000 µL of TRizol used for lysis and mix thoroughly.



5 Incubate for  00:03:00  On ice



3m

6 Centrifuge the sample at  12000 x g, 4°C,
00:15:00





15m

7 Transfer the aqueous phase containing the RNA to a new tube.




Note

Avoid penetration of the interphase layer.

8 Add  500 μL of **Isopropanol** to the aqueous phase, per  1000 μL of TRIzol used.



9 Add  4 μL of **Glycoblue** and mix well.



10 Incubate at  -4 $^{\circ}\text{C}$ for  01:00:00



1h

11 Centrifuge at  30000 x g, 4 $^{\circ}\text{C}$,
00:15:00



15m

Expected result


Blue pellet should be present at the bottom of the tube.

12 Discard supernatant carefully.




13 Resuspend the pellet in  1000 μL of  On ice 75% ethanol per  1000 μL of TRIzol.

5m

Vortex and centrifuge at  7500 x g, 4 $^{\circ}\text{C}$,
00:05:00



14 Repeat **Step 13**.


15 Air dry the RNA pellet for  00:05:00

5m

16 Resuspend the pellet in  20-50 μL of RNase free water.



Note

 40 μL of RNase free water is recommended.

17 Quantify the sample by NanoDrop

