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Retina and RPE/Choroid RNA Extraction Protocol

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Disclaimer

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.

Troubleshooting

Safety warnings

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

Before start

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 °C for 01:00:00 1h

11 Centrifuge at 30000 x g, 4°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. Vortex and centrifuge at 7500 x g, 4°C, 00:05:00 5m

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