

Revised 7/1/2005 Hirotsugu Suwanai

<RNA extraction from frozen spleens by Trizol in CBDM lab>

1. Put 1ml of Trizol per 50-100mg tissue
2. Bring samples in dry ice. Homogenize them with the power homogenizer as soon as you put the frozen tissue into Trizol.
3. Incubate homogenized samples for 5min at R/T.
4. Add 0.2ml of chloroform/1ml Trizol.
5. Shake for 15 seconds and incubate them at R/T for 2 to 3 min.
6. Centrifuge them at 11200rpm(12000xg) for 15 min at 4C.
7. Transfer the aqueous phase to a fresh tube (Max 600ul). Add 0.6ml of isopropyl alcohol per 1ml Trizol.
8. Incubate them at R/T for 10 min and centrifuge at 11200rpm (12000xg) for 10 min.
9. Remove supernate. Wash pellet with 1m 75% ethanol/1ml Trizol. Vortex them. Centrifuge at 9000rpm (7500xg) for 5 min at 4C.
10. Dissolve them with 100-200ul DEPC H2O. Make sure they dissolve completely. You can distribute into some tubes to make aliquots. Put samples into dry ice ASAP.

Usually you would extract 200-300ug of RNA per spleen.