RNA extraction

1. Add 0.3- 0.5 ml of trizol according to the number cells.

2- Or blood, any tissue suspend it with trizol 0.65 ml

3. 5-10 minutes incubation to permit complete dissociation of the nucleoproteins complex .

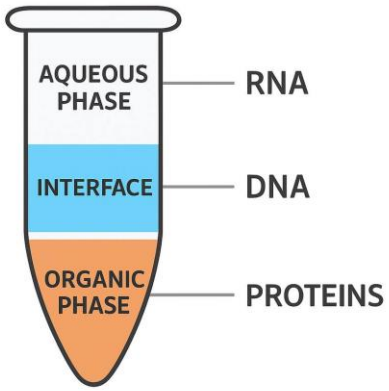
4. The chloroform was added as 250 µl .

5. Incubation for 2–3 minutes at room temperature.

6. The sample was centrifuged for 2-10 minutes at 10,000 rpm. The mixture was separated into a lower organic phase, interphase, and a colorless upper aqueous phase.

7. The aqueous phase containing the RNA transferred to a new tube .

8. The RNA was precipitated by adding 250 µl of isopropanol or absolute ethanol to the aqueous phase.



9. The mixture incubated for 2 minutes at room temperature and transfer to spin column.

10. The mixture was centrifuged for 10 minutes at 10,000 rpm. Total RNA precipitate on filter of spin column tube.

11. The supernatant was discarded.

12. The column gets re-suspended by 0.4 ml of washing buffer 1.

13. The tube was centrifuged for 2 minutes at 10000rpm.

14. The supernatant was discarded.

15. The column gets re-suspended by 0.6 ml of washing buffer 2.

16. The tube was centrifuged for 2 minutes at 10000rpm.

17. The supernatant was discarded.

18. The column gets elution by add 30 - 75 µl of nuclease\RNase free water and centrifuged 1 min at 10000 rpm.

17.Total RNA samples stored in deep freezer.

اسئلة ممكن تنطرح اثناء المناقشة:

- ماهو ال trizol؟

  Phenol+ Guanidium thiocyanate

- وظيفة الtrizol

  يكسر الاغشية الخلوية cell lysis + يثبط ال RNase + يحافظ على RNA من التحلل اثناء الاستخلاص.

- وظيفة ال chloroform

وظيفته مشتركة مع الفينول يكون الاطوار الثلاثة حيث يتم جذب البروتينات والدهون للطور العضوي + تثقيل ال DNA ليبقى في الطبقة الوسطى + عزل ال RNA في الطور المائي

الكلوروفورم يقلل من ذوبانية الأحماض النووية ويغيّر قطبية الخليط بحيث:

- ال RNA يصبح أكثر قابلية للذوبان في الماء
- ال DNA والبروتينات تصبح أقل ذوباناً في الماء .

- وظيفة الغسل (step wash)

Wash 1 :إزالة البروتينات +الدهون +الشوائب العضوية + اجزاء خلوية وتثبيت الRNA

Wash 2 : ازالة الاملاح + بقايا كحول الايثانول والكلوروفورم

- spin column

It has an affinity for nucleic acids (DNA or RNA) only

