77619 Phenol – chloroform – isoamyl alcohol mixture

Product Description:
Phase extraction of nucleic acids is pH-dependent. At pH 7.0 and higher, RNA and DNA will be solved in the aqueous phase. Below pH 7.0, DNA will be denatured and precipitate into the organic phase, leaving RNA in the aqueous phase.

Phenol:chloroform cause proteins to become denatured and become soluble in the organic phase or interphase, while nucleic acids remain in the aqueous phase. The addition of chloroform makes the phase more hydrophobic than phenol alone and prevents poly(A)+ RNA from partitioning into the interphase due to formation of insoluble RNA-protein complexes and reduces cleavage of the poly(A) tail. It also enhances the separation of the organic and aqueous phases and thereby increases the recovery of RNA

Note: Working on ice can help pack the interface.

Troubleshooting:
In case of no phase separation:
- Centrifugation 2 minutes at room temperature: no phase separation
- 30 minutes cooling at -18 °C: product will be solid. Let stand for a while at room temperature and perform later a centrifugation at room temperature: now the product shows two phases (ratio organic phase to water phase about 20: 1).

Possible protocol to purify RNA (first incubation at 18C is needed):
1. 1 volume sample and 1 volume of phenol:chloroform:isoamyl alcohol (125:24:1, pH 4.5). Vortex for 1 minute and spin at top speed in a microcentrifuge for 2 minutes.
2. Transfer the upper, aqueous phase to a fresh tube and add 1 volume of chloroform:isoamyl alcohol (24:1). Vortex for 1 minute and centrifugation for 2 minutes at top spin.
3. Transfer the upper, aqueous phase to a fresh tube. Any transferred chloroform can be removed by centrifugation 10 seconds at top spin followed by removal of the bottom phase with a micropipette.
4. Add 0.1 volume of 3M Sodium Acetate (pH 5.2) and 1 volume of isopropanol or 2.5 volumes of 95% ethanol. Mix and place on ice for 2–5 minutes. Spin at top speed in a microcentrifuge for 10 minutes.
5. Carefully remove the supernatant and wash the pellet with 1ml of 70% ethanol. Dry the pellet under vacuum and resuspend the RNA sample in TE buffer or Nuclease-Free Water to a volume identical to that of the transcription reaction. Store at –70°C.

References:
Manoj C., et al., A method for the extraction of high-quality RNA and protein from single small samples of arteries and veins preserved in RNAlater, Journal of Pharmacological and Toxicological Methods, Volume 47, Issue 2, March-April 2002, Pages 87-92

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.