PanReac AppliChem
ITW Reagents

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Nucleic Acid and Protein Purification with TRItidy G™

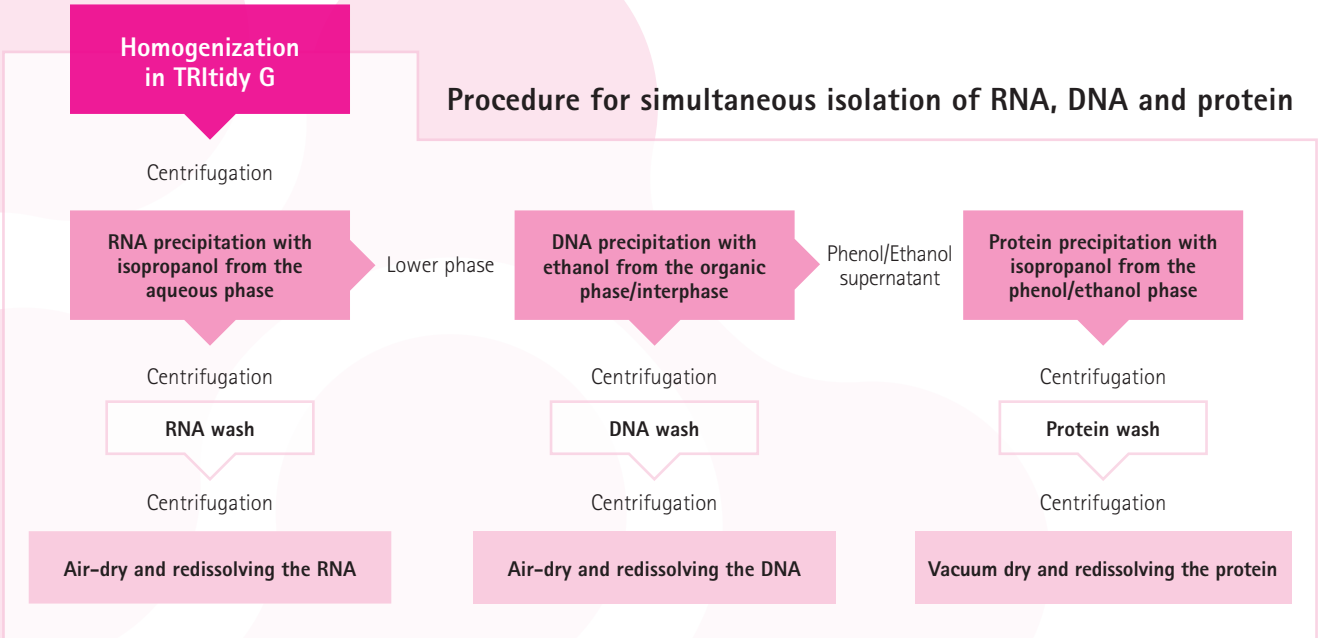
Simultaneous isolation of RNA, DNA and proteins from biological samples was firstly introduced in 1993, based on the use of a reagent containing phenol and guanidine thiocyanate. The simultaneously isolated RNA, DNA and proteins are ready for Northern, Southern and Western blotting as well as PCR, RT-PCR enzymatic assays. The complete recovery of DNA from samples used for the RNA and protein isolation makes it possible to normalize the results of gene expression studies based on DNA content instead of on the more variable total RNA, protein content or tissue weight.

TRItidy G™ is a monophasic reagent, based on the Chomczynski method, with additional modifications to improve the purity of the RNA, DNA and proteins. First, the RNA is selectively retained in the aqueous phase during the acidic extraction, while DNA and proteins stay in the organic phase and interphase, respectively. The DNA is isolated from the interphase/organic phase by a simple ethanol precipitation and proteins from the remaining organic phase.

Main Advantages

- **TRItidy G™** allows **one-step isolation** from the biological sample.
- **Monophasic reagent**
- No need of purification **columns** for the isolation of nucleic acids and proteins.
- **Quick** procedure.
- **Easy** to reproduce.
- Suitable for small and large samples (human, animal, plant, bacterial).

Procedure for simultaneous isolation of RNA, DNA and protein



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Preparation of samples

Depending on your sample type, homogenization of samples should be performed according to the protocol below. The volume of the sample must not exceed 1/10 of the volume of **TRItidy G™**.

Sample type	Procedure
Tissue	Tissue is homogenized in approx. 1 ml **TRItidy G™** per 50 - 100 mg tissue
Cell culture cells (growing in monolayer)	Cells are lysed in 1 ml/10 cm2 (3.5 cm diameter) dish, after aspiration of the medium
Suspension cells	Cells have to be collected by centrifugation before addition of the reagent (1 ml **TRItidy G™** per 1-5 x 10^6 cells; bacteria up to 1 x 10^7).
Blood samples, serum or other biological fluids*	Add 750 µl of **TRItidy G™** per 250 µl of sample volume.

* Biological fluids with high levels of protein or other contaminating substances (e.g. whole blood) may be diluted 1:1 with RNase-free, molecular biology grade water (Suggested product: **A7398**).

Phase separation

Homogenization of the sample → Incubation time (5 min) → Add 0.2 ml of chloroform to the lysate → Additional incubation of 10 minutes → Centrifuge the lysate at 12 000 g for 15 min at 4°C

Purification protocol for RNA, DNA and PROTEINS

RNA Isolation

1. Transfer the aqueous phase to a new tube.
2. Add 1:1 isopropanol.
3. Precipitate the RNA on ice (15 min) and centrifuge.
4. Wash and air-dry RNA.
5. Dissolve in 20 µl DEPC-treated water.

DNA Isolation

1. Add ethanol and incubate (5 min).
2. Centrifuge and remove the supernatant (protein).
3. Wash with sodium citrate 0.1M and centrifuge (5 min).
4. Air-dry the DNA and dissolve in approx. 0.5 ml 1X TE.

Protein Isolation

1. Add isopropanol to the supernatant (2:1).
2. Centrifuge (10 min).
3. Wash protein precipitate with guanidine hydrochloride 0,3M and centrifuge (5 min).
4. Air-dry the precipitate and dissolve in 1% SDS.

Ordering information

Description	Code	Package
TRItidy G™	A4051,0100	100 ml
	A4051,0200	200 ml

Caution: **TRItidy G™** contains Phenol and Guanidinium thiocyanate. For safety instructions please read the Material Safety Data Sheet (MSDS) before use.

Related products

Description	Code
Chloroform BioChemica	A3691
DEPC BioChemica	A0881
Ethanol absolute for molecular biology	A3678
Guanidine Hydrochloride for molecular biology	A1106
2-Propanol BioChemica	A3465
SDS for molecular biology	A2263
TE buffer (1X) pH 7.4 for molecular biology	A9031
Water for molecular biology	A7398

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