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# PhaseAll™ RNA Isolation Protocol

## Product description

PhaseAll™ extraction reagent is an organic phenol-based solution that is ready to use for nucleic acid and protein extraction from human, animal, plant, fungal and bacterial cells and tissues.

Samples are lysed by addition of PhaseAll™ extraction solution. Addition of chloroform allows for separation into an upper aqueous phase containing RNA, a lower organic phase containing denatured proteins, and the dividing interphase, which contains DNA. Separate precipitation and washing steps for each of these three phases enables the extraction of all these three major macromolecules from a single sample.

Extracted macromolecules can be used as follows:

- **RNA** - cDNA synthesis for cloning, RT-PCR, (one-step) qPCR, or RNASeq libary preparation, miRNA detection, northern blot analysis, RNase protection assays, in vitro translation, poly(A) enrichment and most other RNA applications.
- **DNA** - (q)PCR, restriction enzyme digestion, Southern blot analysis, NGS library preparation.
- **Proteins** - western bloting, SDS-PAGE, some immunoprecipitation assays (to be verified case by case).

| Component | 100 extractions |
|---|---|
| PhaseAll™ | 100 mL |

## Shipping and storage

On arrival the kit should be stored between 2 °C and 8 °C in the dark. If stored correctly, the kit will retain full activity until the indicated expiry date.

## Technical support

Scan or click the QR code for answers to frequently asked technical questions including recommended amounts for sample types not listed in the table.

For further technical support, please email technical@pcrbio.com with your query.

FAQS

Tissues for RNA extraction should be processed immediately upon collection, or flash-frozen in liquid nitrogen and stored at -80 °C.

| Sample Type | Amount per 1 mL PhaseAll™ | Notes |
|---|---|---|
| Fresh tissues | 50-100 mg | |
| Cells grown in sigle layer | $10^5$ - $10^7$ cells | |
| Cells grown in suspension | 5-10 x $10^6$ eukaryotic, $10^7$ bacterial cells | |
| Plant tissues (homogenised) | 50-100 mg leaves, 100-200 mg roots | Refer to literature for other tissue types |
| Saliva/Buccal swabs | 100-500 µL | |
| Blood | 100-200 µL blood in anticoagulant | |
| FFPE tissue | 50-100 mg of 5 µM sections (microtome or scalpel) | Separate detailed protocol available |
| Other sample types | Test varying amounts starting at 100 mg/$10^6$ cells | |

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at https://pcrbio.com/terms-conditions/.

# Protocol

## A. Preparing for extraction

- All steps should be performed at room temperature (20-25 ˚C), unless otherwise specified.
- Wear disposable gloves and appropriate protective attire.
- Use aseptic technique to prevent RNA sample contamination with nucleases.
- Use cold PhaseAll™ (from 4 ˚C storage) in the initial extraction step.
- Using an extractor fan/chemical fume hood is recommended, follow your laboratory health and safety guidelines for handling and disposal of phenol and other toxic chemicals.
- Additional materials required: water bath or heat block, centrifuge, microcentrifuge tubes, chloroform, isopropanol, 75% ethanol, RNase-free water or 0.5% SDS, RNase-free glycogen (*optional*).

## B. Tissue preparation, lysis and phase separation

1. Different starting materials require specific handling during this phase. In all cases, sample volume should be ≤0.25 volume of PhaseAll added.
   - **Tissues:** Add 1 mL PhaseAll™ per 50-100 mg, and homogenise. Plant tissues should be ground and homogenised to a fine powder under liquid nitrogen, prior to addition of PhaseAll™.
   - **Cells in single layer:** Remove growth medium, add 1 mL PhaseAll™ per $10^5$ - $10^7$ cells directly to the culture dish and pipette up and down to homogenise. Do not wash cells prior to PhaseAll™ addition.
   - **Cells in suspension:** Collect cells by centrifugation, remove supernatant (culture media) using a pipet, add 1 mL PhaseAll™ per 250 µL of sample ($10^6$ cells). Lyse and homogenise by pipeting up and down. Do not wash cells prior to PhaseAll™ addition.

**Pause point:** At this stage samples can be stored at 4 ˚C overnight, or at -20 ˚C for one year.

2. *Optional step:* For samples with a high fat/oil content, centrifuge lysate for 5 minutes at 12 000 x *g* at 4 ˚C, transfer the clear supernatant to a new tube.
3. Incubate sample for 5 min at room temperature.
4. Add 0.2 mL chloroform (purchased separately) per mL of PhaseAll™, close the tube and vortex thoroughly.
5. Centrifuge at 12 000 x *g* for 15 min at 4 ˚C.
6. For RNA, carefully transfer the aqueous (upper) phase to a new tube for RNA isolation. Continue with RNA isolation in step 7. Do not disturb the interphase or carry over organic phase. For DNA and proteins, remove aqueous phase (save in a separate tube for RNA or discard if unneeded). Follow our corresponding separate protocols for isolation. The organic phase may be stored overnight at 4 ˚C.

## C. Isolating RNA

7. *Optional step:* If input sample is small and RNA expected to be low in quantity, add 5-10 µg RNase-free glycogen to facilitate RNA precipitation. Glycogen does not interfere with downstream RNA reactions.
8. Add 0.5 mL isopropanol per mL of PhaseAll™ used for lysis to the aqueous phase.
9. Incubate for 10 min at room temperature.
10. Centrifuge 10 min at 12 000 x *g* at 4 ˚C.
11. Discard the supernatant with a pipete (do not dislodge any visible RNA pellet).
12. Wash the pellet with 1 mL of 70-75% ethanol per mL of PhaseAll™ used for initial lysis.

**Pause point:** RNA can be stored in ethanol for at least 1 year at -20 ˚C

13. Centrifuge for 5 min at 12 000 x *g* at 4 ˚C
14. Discard supernatant, air-dry pellet at room temperature
15. Resuspend in 30-100 µL RNase-free water (cat. no. PB40.40) or 0.5% SDS.