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PhaseAll™ DNA 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

If tissues will also be used for RNA extraction, they 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 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: centrifuge, microcentrifuge tubes, chloroform, ethanol, sodium citrate (0.1 M) in 10% ethanol (pH 8.5), 8 mM NaOH.

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 DNA and proteins, remove aqueous phase (save in separate tube for RNA extraction, if needed). Follow corresponding separate RNA isolation protocol. The organic phase may be stored overnight at 4 ˚C.

C. Isolating DNA

7. Remove any residual aqueous phase (important for DNA quality).
8. Add 0.3 mL 100% ethanol per mL of PhaseAll™ used for lysis and mix by inverting multiple times.
9. Incubate 3 min at room temperature.
10. Centrifuge 5 min at 2 000 x *g* at 4 ˚C. This precipitates the DNA.
11. Transfer the supernatant (phenol/ethanol mix) to a new tube for protein extraction or discard if unneeded. Follow corresponding separate Protein isolation protocol.
12. Resuspend the pelleted DNA in 1 mL sodium citrate (0.1 M) in 10% ethanol (pH 8.5).
13. Incubate at room temperature for 30 min, gently turning the tube to mix during the incubation.
14. Centrifuge again as in step 10 and discard the resulting supernatant. Repeat steps 12-14 once or twice.
15. Resuspend the pellet in 1.5 mL 75% ethanol.

Pause point: DNA can be stored in ethanol for an extended period at 4 ˚C.

16. Incubate at room temperature for 15 min at room temperature.
17. Centrifuge for 5 min at 2 000 x *g* at 4 ˚C, discard supernatant.
18. Air-dry the pellet and resuspend in 0.3-0.6 mL 8 mM NaOH (a mild base allows easier DNA resuspension).
19. If needed, centrifuge 10 min at 12 000 x *g* at 4 ˚C to remove insoluble residue and transfer the supernatant containing DNA to a new tube.
20. Use directly, or equilibrate pH to 7-8 with HEPES or similar buffer and supplement EDTA to a final concentration of 1 mM for long-term storage.