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DEP-25 DNA Extraction Kit

(Catalogue number D225, D226, D227) rev. 02/2022

Description

DNA **E**xtraction for **P**CR under **25** min (**DEP-25**) is a two-component reagent kit for extraction of genomic DNA of various origin. A major advantage of the kit is that DNA extraction is performed rapidly and is carried out in a single tube - mitigating chances for sample cross-contamination for subsequent PCR analysis. The kit is amenable to high throughput-based screening and is suitable to several downstream applications such as identification of genotypes, DNA fingerprinting, and cell identity/contamination analysis.

Rapid, easy to use, robust, safe and reliable DNA extraction

- DNA from various sources can be prepared in 25 min or less; no need for lengthy enzymatic digestion, column purification or phenol/chloroform extraction **(Fig. 1)**. Suitability of the extracted DNA for PCR analysis has been determined by testing PCR amplification of various targets **(Fig. 2)**.

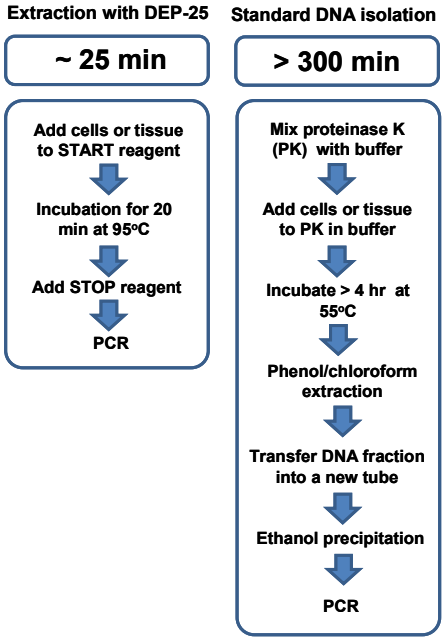

Fig. 1. Schematic presentation of two methods used for preparation of genomic DNA in quality suitable for PCR analysis. Extraction with DEP-25 consists of 3 simple steps and lasts ~25 minutes. This procedure is shorter, simpler and cheaper when compared to DNA isolation using standard DNA isolation method based on proteinase K digestion and phenol/chloroform extraction.

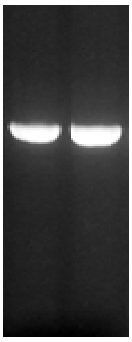

Fig. 2. PCR amplification of genomic DNA extracted with DEP-25 (**1**) or isolated by standard DNA isolation method (**2**). For PCR with Combi PPP Master Mix, oligonucleotide primers specific for 864 bps fragment were used [Nucl. Acids Res., 36 (15):e93, 2008]. PCR amplicons were size-fractionated by agarose gel and stained with ethidium bromide. Only fragments of the expected size were visible.

Color indicator

- DEP-25 is supplied as a two-component kit **START-Blue** & **STOP**. START-Blue is a color indicator that provides visual identification of proper solution used for DNA extraction. Upon addition of equal volume of STOP reagents, solution turns colorless.

Complete package

- DEP-25 contains ready to use reagents needed for rapid extraction of DNA from variety of tissues, including cell lines of different origin, tissue samples (hair, buccal cells, saliva) mouse tail biopsies or ear punches.

Versatile

- DNA extracted with DEP-25 is compatible with both endpoint PCR and real-time qPCR. The extracted DNA is compatible as a template with all Master Mixes of TB provenience.

Economical

- The kit provides enough reagents sufficient for DNA extraction at price, which is a fraction of the price when other protocols are used.

Cat. No.	Product Name and Specification	Amount
D225	DEP-25 DNA Extraction Kit, **100** extractions	2x 8 ml
D226	DEP-25 DNA Extraction Kit, **400** extractions	2x 30 ml
D227	DEP-25 DNA Extraction Kit, **1600** extractions	2x 120 ml

Technical Data

Components and Packaging

- DEP-25 DNA extraction Kit is supplied in bottles containing either 2x 8 ml (Cat. No. D225), 2x 30 ml (Cat. No. D226) or 2x 120 ml (D227) of START-Blue and STOP reagents.
- Each DEP-25 kit contains detailed protocol for DNA extraction.

Storage

- At temperature 2 - 8°C. For short time (days) at temperature up to 35°C. This allows transport without cooling (nature friendly).

Purity and Quality Control

- Each batch of DEP-25 is tested for its ability to extract genomic DNA, which can be amplified by PCR. The results are verified by electrophoresis in agarose gel in the presence of ethidium bromide; only DNA band of the expected size is present **(Fig. 2)**.

Protocol

Reagents and Equipment Required but not Provided

- Microcentrifuge tubes (0.2 ml or 0.5 ml), PCR tubes (0.2 ml), or 96-well PCR plates.
- Pipeting devices and tips.
- Thermal cycler (95^{o}C) or heat block for 0.2 or 0.5 ml tubes or a 96 -well plate.

All steps are carried out at room temperature unless otherwise stated.

Procedure

1. Spin down 10^5 - 10^6 cells in 0.2 ml or 0.5 ml test tube and discard the supernatant. Alternatively, transfer piece of tissue (e.g. 2 - 5 mm of frozen or fresh mouse tail, frozen or fresh mouse ear punches, hair shafts, or 2 - 10 mg of selected tissue) into test tubes.
2. Add 75 µl of DEP-25 START-Blue reagent. Ensure that tissue or cells are completely submerged in the START-Blue solution. When foam buccal swabs are used, immerse them into the 0.2 ml PCR tube with 75 µl of DEP-25 START-Blue reagent and then twirl the swab in the START-Blue reagent.
3. Heat samples to 95^{o}C for 20 min. PCR cycler or heat block can be used for this step. Solid tissues will not be completely soluble at the end of incubation. This is normal and will not affect performance of the extracts in PCR.
4. Cool samples to room temperature, add 75 µl of DEP-25 STOP solution and mix by vortexing. Upon addition of STOP solution START-Blue reagent should turn colorless.
5. Use 1 - 2.5 µl of the extract as a source of DNA template for 25 µl PCR or qPCR. When scaling PCR reactions, add ≤ 1/10 volume of undiluted DNA extract to the PCR.

Protocol Notes

Storage. DNA extracts can be stored at 4 ± 2^{o}C or frozen at -20 ± 5^{o}C for several months. It is not necessary to remove residual tissue from the extracts.

High throughput. Cell or tissue extraction with DEP-25 START Blue reagent can also be done in 96-well plates for PCR and heated at 95^{o}C in PCR machines.

Faster protocol. Extraction incubation time can be shortened to 10 minutes at 95^{o}C. When cells from cell culture are used, 5 min incubation can suffice.

Maximum yield. Tissue can be divided into smaller pieces to expose more surface area to the START-Blue reagent resulting in shorter extraction time and/or greater yield of extracted DNA. Extraction incubation time can be extended to 60 minutes at 95^{O}C. The yield of extracted DNA will generally increase with increased incubation time. Optimal extraction incubation time will depend on the tissue sample.

DNA extraction from buccal swabs. Due to the low volume of solution used for DNA extraction, a small foam tipped swab should be used. Swabs with fibrous tips, such as cotton or Dacron, should be avoided because the solution cannot be recovered efficiently.

Scale up/down. DNA Extraction can be performed in different vessels and different volumes; however, equal volumes of START-Blue and STOP solutions should is used and final solution with DNA extract must be colorless.

Suboptimal PCR performance. If large number of cells or amount of tissue is used, DNA extract may contain PCR inhibitors. Reducing amount of template per PCR or dilution of the extract in PCR water should solve the problem. If low number of cells is used number of PCR cycles should be extended.