Tissue Ex-Amp PCR Kit (with Kodaq MasterMix)

Store at -20°C

Cat. No.	Description	Quantity
G927	Tissue Ex-Amp PCR Kit (with Kodaq MasterMix)	100 preps, 120 x 50 µl rxn

Product Description

crb's Tissue Ex-Amp PCR Kit offers the ultimate convenience for gDNA PCR from tissues, cells or whole blood. This advanced and proprietary direct gDNA extraction from raw samples only involves the use of one single extraction buffer, eliminating the need of neutralizing the crude lysate as well as the trouble of re-opening the sample tube during the extraction process, thus minimizing the risk of contamination and human error. It only takes 15 easy minutes to "incubate and boil", and the processes can be carried out in a water bath or with the convenience of a thermal cycler. The kit comes with **crb**'s sophisticated Kodaq 2X PCR MasterMix with dye; the MasterMix offers an un-beatable robustness and extreme fidelity in a streamlined and efficient PCR setup.

Kodaq 2X PCR MasterMix with dye contains a green dye blend which resolves during gel electrophoresis into a turquoise band at ~4000bp and a yellow band at the ~50bp region. The resolving bands of dye allow easy visualization of the real time progress of your gel electrophoresis, where the yellow band indicates the migrating front in the gel.

Features and Benefits

- No time-consuming DNA purification steps.
- No neutralization step needed.
- Only small amount of sample needed.
- Simple universal protocol for various tissue or cell samples.
- Kodaq 2X PCR MasterMix with dye for robust and gel-loading-ready PCR.

Part No.	Product Components	Quantity
G927	Tissue Ex-Amp Extraction Buffer	6 X 1.25 ml
G497-dye	Kodaq 2X PCR MasterMix with dye	3 X 1 ml

Shipping and Storage

Keep at -20°C for long term storage; MasterMix may not freeze at -20°C.
A small amount of salt precipitation may occur after thawing but can be re-dissolved into the MasterMix by mixing well. Kodaq 2X PCR MasterMix with dye is stable at 4°C for one month or at least fifteen freeze-thaw cycles (-80°C). For daily use, we recommend keeping an aliquot at 4°C.

Protocol

PCR reactions should be assembled in a nuclease-free environment. DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction analysis, should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended. Always keep the control DNA and other templates to be amplified isolated from the other components.

A control reaction, omitting template DNA, should always be performed to confirm the absence of contamination.

DNA Extraction:

E1. Place a piece of tissue with 1 - 2 mm in diameter, or a 10_3 - 10_5 cells pellet, or 5 - 10 µl of whole blood sample into an PCR tube.
E2. Add 75 µl of the Tissue Ex-Amp Extraction Buffer to the sample tube and gently mix the content.
E3. Using a thermal cycler, incubate the extraction tube first at 55°C for 10 minutes (longer incubation time upto 2 hours can also be used) and then at 95°C for 5 minutes. (Thermal cycler can be replaced by hot water baths or heating blocks.)
E4. Chill the extraction mixture on ice briefly; the solution now can serve as template in PCR reactions.

PCR Amplification:

A1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final Concentration
Template DNA (From Step E4)	1 - 2 µl	Varies
Forward primer (10 µM)	1 - 2.5 µl	0.2 - 0.5 µM
Reverse primer (10 µM)	1 - 2.5 µl	0.2 - 0.5 µM
Kodaq 2X PCR MasterMix with dye	25 µl	1X
Nuclease-free H_2O	up to 50 µl	

- *We recommend preparing a pre-mix for multiple reactions to minimize reagent loss and enable accurate pipetting.*

A2. Mix contents of tube and centrifuge briefly.
A3. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the template.
A4. Perform 30 - 40 cycles of PCR amplification as follows:

Denature:	94°C for 30 secs
Anneal:	45°C - 72°C for 30 secs
Extend:	72°C for 1 min/1 kb template

A5. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
A6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ (Cat No. G108) staining. Since Kodaq 2X PCR MasterMix with dye is used, load the samples directly without adding additional loading dye. Use appropriate molecular weight standards.

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