شرکت تحقیقاتی تولیدی پویا ژن آزما

POUYA GENE AZMA Ltd. (PGA)

PGA Tissue and cell culture DNA Extraction KIT

Catalog No. PD130-050 50 Preps
Store at: RT

For research use only

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KIT CONTENTS:

Buffer MI	10 ml
Buffer MII	3 ml
Buffer MIII	5 ml
Solvent Buffer	3 ml
Proteinase K	100 µl
RNase A	35 µl

Required contents: **Cold ethanol %100**
Cold ethanol %70

KIT Description:
The PGA Tissue and Cell culture DNA Extraction KIT contains all the ingredients for quick preparation of genomic, mitochondrial, or viral DNA from cell cultures or animal tissues. The high-quality extracted DNA procedure is ready to use in molecular biology procedures (PCR, restriction digestion, cloning, Southern blot, DNA sequencing, etc.). The KIT is safe and user-friendly with no need for phenol and chloroform.

LABORATORY PROTOCOL:

Attention: Please add RNase A to MI Buffer before use and keep at 4℃.

Before start:
-Set a water bath at 55℃
-Preparation of "Ready to use" MI buffer: Mix 200 µl MI buffer (pre-mixed with RNase) with 2 µl Proteinase K per sample. Scale up the volume for multiple samples.

Tissue and cell culture dissociation

- For Tissue dissociation: Transfer 10-20 ng of tissue to a 1.5 ml microtube, use micropestle to grind several times.
- For cell dissociation: Transfer 200-500 µl of pop smear suspension or 3-5 × 10^6 cell culture to a 1.5 ml microtube, centrifuge 5 minutes at maximum speed. Remove the supernatant and keep the precipitate.

1. Add 200 µl "Ready to use" MI buffer to re-suspend the precipitate or the tissue sample. Mix thoroughly and incubate at 55℃ for 30-50 minutes (mix occasionally to aid digestion).
2. Add 50 µl MII buffer to the tube and gently invert 2 times. Keep it at room temperature for 5-10 minutes until a clear solution is produced.
3. Add 1 µl of RNase A to the lysate, mix by pipetting 5 times or until a homogenous solution is obtained. Incubate at RT for 5 minutes.
4. Add 100 µl MIII buffer to the tube and invert 10 times. (When MIII buffer is added, the white precipitate is produced).
5. Microfuge at 13000 rpm for 10 minutes.
6. Transfer the supernatant to a new tube. (Important: Do not transfer the precipitate to the new tube. In this case, repeat steps 5 and 6.)
7. Add 2 volumes of solution, cold ethanol 96%-100% to the solution and invert 5 times. Microfuge at 13000 rpm for 5 minutes.
8. Pour off ethanol and wash the precipitate by adding 400 µl cold ethanol %70 and invert 2-3 times.
9. Microfuge at 13000 rpm for 2-3 minute.
10. Pour off the ethanol completely and dry the pellet for 2-3 minutes at room temperature.
11. According to the precipitate, add 20-50 µl Solvent Buffer. The precipitate must be solved completely.

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