A&A BIOTECHNOLOGY
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Manual

Fast DNA Plant Screen PCR

Kit for rapid isolation of genomic DNA from plant material, to be used in PCR.

catalog #	size
050-192P	192 isolations (2 x 96)

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of Contents

Contents

component	quantity	storage
A buffer	5 x 4 ml	-20 °C
B buffer	5 x 4 ml	-20 °C
PCR Mix Plus HGC	1 pcs	-20 °C

Additional equipment and reagents

Necessary

- 1,5 ml Eppendorf tubes
- Heatblock or incubator set to 95 °C

Optional

- PCR tubes or 96-well PCR plates

Isolation protocol

1. Cut the plant tissue in small fragments (size not exceeding 2-3 mm) and transfer to 1,5 ml Eppendorf tubes (not included).
2. Add **50-100 µl** of **A** buffer. Mix by pipetting. Sample must be completely submerged in A buffer.
3. Incubate the sample for **10 min** at **95 °C**.
4. Cool down the samples to **room temp**.
5. Add an equal volume of **B** buffer (about **50-100 µl**).
6. Mix the samples for **5 s** by pipetting or vortexing.
7. Store the samples with fragments of plant tissue up to **3 months** at **4-8 °C**.

   Do not freeze!
   Freezing may cause DNA degradation.

We recommend using isolated DNA to maximum 10% of the final volume of PCR sample (e.g. 5 µl of isolated DNA to 50 µl of final volume of PCR mix reaction).

Example PCR protocol

We recommend using isolated DNA to maximum 10% of the final volume of PCR sample (e.g. 5 µl of isolated DNA to 50 µl of final volume of PCR mix reaction).

- Before use all solutions should be thawed thoroughly on ice, gently mixed by inverting the tube and briefly centrifuged.
- Up to 7x repeated freeze-thaw cycles do not influence the activity of this product.

1. Thaw **the PCR Mix Plus HGC** and **ultrapure water** on ice, gently mix by inverting the tubes and briefly centrifuge. Place the tubes on ice again.
2. Place PCR tubes on ice and add:

	PCR reaction volume	
component	**25 µl**	**50 µl**
PCR Mix Plus HGC	12.5 µl	25 µl
Starter 1	0.1-1 µM	0.1-1 µM
Starter 2	0.1-1 µM	0.1-1 µM
DNA template	10 pg-1 µg	10 pg-1 µg
Ultrapure water	up to 25 µl	up to 50 µl

3. Gently mix the samples and briefly centrifuge. If necessary, overlay the samples with mineral oil. (it's recommended for thermocyclers without a heated lid).
4. Place the tubes in the thermocycler and start the PCR programme.

   An example amplification profile for products up to 500 bp:

step	**temperature**	**time**
Initial denaturation	95 °C	2-3 min
25 - 45 cycles	95 °C 50 - 68 °C 72 °C	15-30 s 30-60 s 15-60 s

5. Load the post-PCR samples directly on an agarose gel for electrophoresis.

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