Extract-Amp RED PCR Kit

Cat #: SMP42-502

Contents: 20 Reactions

Storage: -20°C.

Reagent for *in vitro* laboratory use only

General Description

Apex Extract-Amp RED PCR Kit consists of Apex DNA extraction solution and Apex 2.0X Taq RED Master Mix, which is required for the subsequent PCR.

Apex DNA Extraction Solution is designed for rapid and efficient extraction of PCR-ready DNA from various sample types; mammalian tissues (such as mouse tail and ear snips), plant leaves, saliva, and bacteria. The non-toxic DNA Extraction Solution enables the extraction of DNA from tissues in just 8 minutes. The extraction protocol is divided into two simple heating steps, which can be directly followed by PCR using 2.0X Taq RED Master Mix. This method is ideal for PCR analysis such as screening and genotyping.

The one-reagent set-up is easily scaled and can be conducted by robotic automation platforms. Depending on the sample size, the DNA extraction can be performed in PCR tubes or 1.5 ml tubes using either a thermocycler or heating block. 2.0X Taq RED Master Mix is a ready-to-use 2x reaction mix. Each PCR reaction requires 12.5 µl of the master mix. Simply add primers, DNA extract and water to a total reaction volume of 25 µl to successfully carry out PCR.

There is no need to use separate loading dyes. Simply load a portion of the reaction product onto an agarose gel for electrophoresis and subsequent visualization. The red dye front runs at 1000 – 2000 bp on a 0.5 – 1.5 % agarose gel.

This kit combination allows for DNA extraction and amplification hereof in less than 1½ hour, as compared to ≥1 day with conventional protocols.

Composition of Apex DNA Extraction Solution

- Optimized DNA Extraction Solution

Composition of Apex 2.0X Taq RED Master Mix

- Tris-HCl pH 8.5, (NH4)2S04, 3 mM MgCl2, 0.2% Tween® 20
- 0.4 mM of each dNTP
- **Apex** Taq DNA polymerase
- Inert red dye and stabilizer

Storage and Stability of kit components

Apex DNA Extraction Solution:
Long term storage at -20 °C. Product expiry at -20 °C is stated on the label. **Optional:** Can be stored short term at +4 °C for up to 3 months. The DNA Extraction Solution tolerates up to 20 freeze-thaw cycles. It is recommended to aliquot the DNA Extraction Solution into smaller volumes before use.

Apex 2.0X Taq RED Master Mix:
Long term storage at -20 °C. Product expiry at -20 °C is stated on the label. Can be stored at +4 °C for up to 6 months.

Quality Control

Each batch of **Apex** DNA Extraction Solution is functionally tested.

Apex Taq DNA Polymerase is functionally tested and tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

Extraction Protocol

Preparation of DNA extraction should be performed in a separate area from that used for setting up the PCR reaction.

1. Thaw DNA Extraction Solution. For the first time use, aliquot the DNA Extraction solution into smaller volumes. (DNA Extraction Solution has a cloudy appearance)
2. Add your sample to a tube containing 100 µl DNA Extraction Solution. Recommended sample sizes are shown in Table 1.
3. Vortex the tube containing the sample and the DNA extraction solution for 15 sec. Make sure that the sample is completely covered by the DNA Extraction Solution.
4. Transfer the tube to a heat block or a thermal cycler and incubate for:

    1. 65 °C for 6 min
    2. 98 °C for 2 min
    3. 4 °C (or cool down on ice)

    The DNA extract is now ready for PCR.

DNA extracts are stable at -20 °C for one week or long term at -80 °C.

5. Mix the DNA extract with 2.0X Taq RED Master Mix. See PCR protocol and table 2.

Table 1. Sample sizes

Sample - type	DNA Extraction Solution	
	100 µl	500 µl
Tissue*	0.5 – 10 mg	10 – 50 mg
Plant**	2 – 10 mg	10 – 50 mg
E. coli	1 colony (Φ 0.5 - 2 mm)	1 colony (Φ 0.5 - 5 mm)
Saliva	10 – 20 µl	50 - 100 µl

* Examples of tested tissues include mouse tail snip, mouse organs and chicken breast. **Examples of tested plant materials include leaves from stinging nettle and ivy.

Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

Preprotocol considerations:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- The table below shows the reaction set up for a final volume of 25 µL. If desired, the reaction size may be scaled up or down.
- Keep all components on ice.
- **Important:** Mix the solutions completely before use to avoid localized concentrations of salts.

1. Set up each reaction as follows:

Component	Vol./Reaction	Final Conc.
2.0X Taq RED Master Mix	12.5 µL	1X
Primer A	Variable	0.1–1.0 µM
Primer B	Variable	0.1–1.0 µM
Nuclease-Free Water	Variable	- - - -
DNA	Variable	Variable
TOTAL volume	25 µL	- - - -

2. Mix gently.
3. Add extracted DNA to the individual tubes containing the reaction-mix.
4. Program the thermal cycler according to the manufacturer´s instructions. See table 3 for an example.
5. Place the tubes in the thermal cycler and start the reaction.
6. At the end of the run, simply load a portion of the reaction product (e.g. 10 µl) onto an agarose gel for analysis.

For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

Table 3. Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2-5 minutes	95 °C
25-35	20 – 30 seconds 20-40 seconds 30 seconds	95 °C 55 – 60 °C 72 °C
1	5 minutes	72 °C

Related Products

Extraction Solution (500 reactions)	Cat#
DNA Extraction Solution	42-503B

Genotyping PCR kit (500 reactions)	Cat#
Extract-Amp RED PCR Kit	42-502B

Taq Polymerase kits (500 units)	Cat#
With 10X Standard and Ammonium Reaction Buffer	42-800B1
With 10X Combination Buffer	42-800B3
Glycerol Free	42-800B4

Hot Start DNA Polymerase kit (500 units)	Cat#
With 10X Ammonium and Combination Reaction Buffer	42-106

High Fidelity DNA Polymerase (500 units)	Cat#
With 5X High Fidelity Reaction Buffer	42-500B

Master Mixes (500 reactions)	Cat#
2X Taq RED Master Mix, 1.5 mM $MgCl_2$	42-138
2X Taq Master Mix, Clear, 1.5 mM $MgCl_2$	42-134
2X Hot Start Master Mix Buffer I, 1.5 mM $MgCl_2$	42-198
2X Hot Start Master Mix Buffer I Blue, 1.5 mM $MgCl_2$	42-144
2X High Fidelity Master Mix	42-501B

Real-time PCR (400 reactions)	Cat#
qPCR 2X Master Mix for Probe, without ROX™	42-116P
qPCR 2X Master Mix for Probe, low ROX™	42-118P
qPCR 2X Master Mix for Probe, high ROX™	42-120P
qPCR 2X GREEN Master Mix, without ROX™	42-116PG
qPCR 2X GREEN Master Mix, low ROX™	42-118PG
qPCR 2X GREEN Master Mix, high ROX™	42-120PG

Ultrapure dNTPs	Cat#
dNTP set, 100 mM each: 250 µl of each dA, dC, dG and dT	42-410
dNTP Mix 40 mM (1 x 500 µl): 10 mM each dA, dC, dG, dT	42-411

DNA Ladders	Cat#
Apex 100 bp-Low DNA Ladder, 250 applications	19-109
Apex 1 kb DNA Ladder, 333 applications	19-115
Apex 200 bp DNA Ladder, 200 applications	19-111
ECON Mini DNA Ladder 100-500 bp, 100 applications	19-130
ECON Low DNA Ladder 100-1000 bp, 100 applications	19-131
ECON PCR Ladder 100-3000 bp, 100 applications	19-132

Accessory reagents	Cat#
50 mM MgCl2, 3 × 1.5 ml	42-303
Nuclease-Free Water, PCR Grade, 6 x 5 ml	42-710