KAPA3G Plant PCR Kit

KR0388_S – v3.20

Kit Codes and Components	
KK7251 (250 U) **KK7252** (500 U)	KAPA3G Plant DNA Polymerase (2.5 U/µL) KAPA Plant PCR Buffer (2X) KAPA $MgCl_2$ (25 mM)

Product Description

KAPA3G Plant PCR Kits are designed for PCR of plant-derived DNA, using either purified DNA or DNA prepared by crude extraction methods (crude sample PCR). In addition, the KAPA3G Plant PCR Kit can be used to amplify DNA from plant material added directly to the PCR (direct PCR).

The KAPA3G Plant PCR Kit contains the novel KAPA3G DNA Polymerase, which was engineered via a process of directed evolution for improved tolerance to common plant-derived PCR inhibitors such as polyphenolics and polysaccharides. KAPA3G Plant DNA Polymerase is a blend of the engineered A-family KAPA3G DNA Polymerase and a modified B-family DNA Polymerase. The enzyme blend is combined with proprietary antibodies to inactivate the enzymes prior to the first denaturation step. The fidelity of the KAPA3G Plant DNA Polymerase is 4–10 times higher than that of wild-type *Taq*. A high proportion of DNA fragments generated with KAPA3G Plant PCR Kits are 3'dA-tailed and may be used for routine downstream analysis and applications, including restriction enzyme digestion, TA cloning and sequencing.

The KAPA Plant PCR Buffer (2X) is a ready-to-use mixture of all reaction components except DNA polymerase, primers and template, and contains dNTPs at 0.2 mM each (1X), and $MgCl_2$ at 1.5 mM (1X). The novel buffering system allows specific amplification over a wide range of annealing temperatures, reducing the need for extensive annealing temperature optimization.

This protocol describes three methods for Plant PCR with the KAPA3G Plant PCR Kit: PCR from purified DNA (Section 1), direct PCR (Section 2) and crude sample PCR (Section 3). Reaction conditions should be optimized with purified DNA prior to attempting either direct or crude sample PCR.

Product Applications

The KAPA3G Plant PCR Kit is ideally suited for:

- Amplification of fragments up to 5 kb in size from purified plant DNA, extracted with commercial kits or CTAB-based methods
- Direct PCR from leaf discs, seed samples, and other plant tissue types
- PCR from crude plant DNA extracts, prepared from leaf and/or seed material.

For more information on the optimization of Plant PCR with the KAPA3G Plant PCR Kit, please refer to the **KAPA3G Plant Application Note**, available from sigma-aldrich.com/techservice.

Quick Notes
• KAPA3G Plant DNA Polymerase is tolerant to plant-derived PCR inhibitors, and can amplify from purified DNA, crude extracts, and plant material. • Optimize reaction conditions using purified DNA before attempting direct or crude sample PCR. • For direct PCR, use a sampling tool to control the amount of plant material added to the reaction. The use of excessive amounts of crude plant material in PCR is a major cause of direct PCR reaction failure. • For crude sample PCR, prepare a crude DNA extract using a small amount of plant material in Extraction Buffer (Refer to **Section 3: Crude sample PCR**), and use 1 µL per 50 µL reaction. • KAPA Plant PCR Buffer contains $MgCl_2$ (1.5 mM at 1X) and dNTPs (0.2 mM each at 1X). Additional $MgCl_2$ (25 mM) is supplied separately for optimization.

Product Specifications

Shipping, storage and handling

KAPA3G Plant PCR Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon arrival, store kit components at -15°C to -25°C in a constant-temperature freezer. When stored under these conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label.

Always ensure that the product has been fully thawed and mixed before use. Reagents may be stored at 2°C to 8°C for short-term use (up to 1 month). Return to -15°C to -25°C for long-term storage.

Quality control

Each batch of KAPA3G Plant DNA Polymerase is confirmed to contain <2% contaminating protein (Agilent Protein 230 Assay). KAPA3G Plant PCR Kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activity, and meet strict requirements with respect to DNA contamination levels.

Section 1: PCR from purified DNA

NOTE: Before attempting direct or crude sample PCR, first optimize reaction conditions with purified DNA, using the protocol described below. Once reaction conditions have been optimized, proceed with either direct PCR (**Section 2**) or crude sample PCR (**Section 3**).

Reaction Setup: Purified DNA

Set up each reaction as follows:

Component	50 µL reaction[1]	Final conc.
PCR-grade water	Up to 50 µL	N/A
2X KAPA Plant PCR Buffer[2]	25.0 µL	1X
25 mM $MgCl_2$	As required	>1.5 mM[3]
10 µM Forward Primer	1.5 µL	0.3 µM
10 µM Reverse Primer	1.5 µL	0.3 µM
Template DNA[4]	As required	As required
2.5 U/µL KAPA3G Plant DNA Polymerase[5]	0.4 µL	1 U

[1] Reaction volumes may be scaled down to <50 µL once conditions have been optimized.

[2] 2X KAPA Plant PCR Buffer contains 1.5 mM $MgCl_2$ (1X) and 0.2 mM of each dNTP (1X).

[3] Supplement reactions with additional $MgCl_2$ if required.

[4] Use 1–50 ng of purified DNA per 50 µL reaction.

[5] Use 1 U of enzyme per 50 µL reaction. Amplification from purified DNA is unlikely to require additional enzyme.

Cycling protocol

Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	3 min	1
Denaturation[1]	95°C	20 sec	35
Annealing[2]	50–68°C	15 sec	
Extension	72°C	30 sec/kb	
Final extension	72°C	1 min/kb	1

[1] Denature for 20 sec/cycle. For complex or GC-rich targets, denaturation may be increased to 30 sec/cycle.

[2] Use the average primer T_m + 2°C, or optimize the annealing temperature using gradient PCR.

Section 2: Direct PCR

Direct PCR may be performed using leaves or seeds from a variety of plant species, but is not recommended for plants with a high inhibitor content (e.g. *Eucalyptus* spp.). Controlling the amount of plant material added to the PCR is critical, and a sampling tool (0.35–0.5 mm diameter) should be used to ensure consistency. If too much plant material is added to the PCR, reaction failure is highly likely. If results with direct PCR are poor, or if multiple reactions are to be performed from a single sample, crude sample PCR (**Section 3**) is recommended.

Reaction Setup: Direct PCR

Set up each reaction as follows:

Component	50 µL reaction[1]	Final conc.
PCR-grade water	Up to 50 µL	N/A
2X KAPA Plant PCR Buffer	25.0 µL	1X
25 mM $MgCl_2$	As required	>1.5 mM[2]
10 µM Forward Primer	1.5 µL	0.3 µM
10 µM Reverse Primer	1.5 µL	0.3 µM
TCEP (optional)[3]	As required	0.5–5 mM
Template leaf/seed punch[4]	0.3–0.5 mm diameter	N/A
2.5 U/µL KAPA3G Plant DNA Polymerase[5]	0.4 µL	1 U

[1] Reaction volumes below 50 µL are not recommended, since this results in a high concentration of inhibitors in the reaction.

[2] Direct PCR typically requires higher $MgCl_2$ concentrations. The optimal $MgCl_2$ concentration should be determined empirically, but is generally in the range of 2–3 mM.

[3] Should reactions fail or produce a low yield of amplicon, TCEP (optional; not provided) can be added to a final concentration of up to 5 mM.

[4] Use a sampling tool to add a 0.35–0.5 mm leaf/seed section.

[5] Direct PCR may require additional enzyme. Should 1 U per reaction produce low yields, increase in 1 U increments.

Cycling Protocol

Perform PCR with the cycling protocol that was optimized using purified DNA (**Section 1: PCR from purified DNA**). Please note the following:

- Direct PCR is likely to require a higher number of cycles than PCR from purified DNA. Start with at least 40 cycles, and increase in 5 cycle increments if necessary.
- Direct PCR may require longer extension times to compensate for the presence of inhibitory compounds. Should 30 sec/kb not produce a high yield of product, increase in 15 sec increments.

Section 3: Crude Sample PCR

For crude sample PCR with the KAPA3G Plant PCR Kit, prepare a crude extract using the protocol outlined below.

Extraction Buffer Preparation

Prepare the required volume of KAPA3G Plant Extraction Buffer, consisting of:

- 50 mM Tris-HCl (pH 8.0–8.5)
- 0.1 mM EDTA
- 2% ß-mercaptoethanol (added freshly before use)[1]
- Optional: 1 mM TCEP[2]

1. Dithiothreitol (DTT) may be used at a final concentration of 10 mM as an alternative should ß-mercaptoethanol not be available. Extracts prepared with DTT are typically less stable than those prepared with ß-mercaptoethanol, and produce lower yields in PCR.
2. Use 1 mM TCEP as an additive only if the Extraction Buffer does not work well without it. Extraction Buffer with and without TCEP may be evaluated to determine if it is required for your particular sample type.

Extraction Procedure

The optimal extraction procedure for a particular plant type should be determined when first attempting crude sample PCR. This involves deciding whether a heat treatment step is necessary or not, and is done as follows:

- Cut two ~5 x 5 mm pieces of leaf or seed using either a scalpel or a single-hole punch, and place into two microcentrifuge tubes. For seeds smaller than 5 mm, use a whole seed, crushed or cut so that the cotyledon is exposed. Larger seeds, or seeds with hard exteriors, may have to be cut with a scalpel, or ground with a mortar and pestle.
- Add 100 µL (or more, if required) Extraction Buffer to each tube, ensuring that the entire sample is submerged in the buffer.
- Use a sterile pipette tip to bruise leaf/seed samples gently in the Extraction Buffer.
- Place one of the two tubes on ice, and incubate the other at 95°C for 5 minutes. Once the incubation is complete, place the tube on ice until PCR setup.
- Perform PCR from each of the two crude extracts to determine whether your plant type requires a heat treatment or not.

The stability of an extract prepared from a particular sample type should be determined empirically. Extracts prepared with this protocol using Extraction Buffer with ß-mercaptoethanol are typically stable for 3–5 days at 2°C to 8°C. Extracts prepared using Extraction Buffer with DTT are generally less stable, so DTT should only be used if ß-mercaptoethanol is not an option.

Reaction Setup: Crude sample PCR

Set up each reaction as follows:

Component	50 µL reaction[1]	Final conc.
PCR-grade water	Up to 50 µL	N/A
2X KAPA Plant PCR Buffer	25.0 µL	1X
25 mM $MgCl_2$	As required	>1.5 mM[2]
10 µM Forward Primer	1.5 µL	0.3 µM
10 µM Reverse Primer	1.5 µL	0.3 µM
TCEP (optional)[3]	As required	0.5–5 mM
Crude extract	1.5 µL	N/A
2.5 U/µL KAPA3G Plant DNA Polymerase[4]	0.4 µL	1 U

[1] For initial validation, use 50 µL reactions. To scale down, refer to **Important Parameters: Reaction volume**.

[2] Crude sample PCR typically requires higher $MgCl_2$ concentrations. The optimal $MgCl_2$ concentration should be determined empirically, but is generally in the range of 2–3 mM.

[3] Should reactions fail or produce a low yield of amplicon, TCEP (optional; not provided) can be added to a final concentration of up to 5 mM.

[4] Crude sample PCR may require additional enzyme. Should 1 U per reaction produce low yields, increase enzyme in 1 U increments.

Cycling Protocol

Perform PCR with the cycling protocol that was optimized using purified DNA (**Section 1: PCR from purified DNA**). Please note the following:

- Crude sample PCR is likely to require a higher number of cycles than PCR from purified DNA. Start with at least 40 cycles, and increase in 5 cycle increments if necessary.
- Crude sample PCR may require longer extension times to compensate for the presence of inhibitory compounds. Should 30 sec/kb not produce a high yield of product, increase in 15 sec increments.

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at www.sigmaaldrich.com, or upon request from www.sigma-aldrich.com/techservice.

Important Parameters

Reaction volume

Reaction optimization should be performed in 50 µL volumes. If using purified DNA as template, reaction volumes may be scaled down as required. The crude sample PCR protocol (**Section 3**) has been validated with 10 µL reaction volumes (using 1 µL of a 1:10 dilution of crude extract per reaction). For direct PCR, reaction volumes <50 µL are likely to result in reaction failure, unless the sample size can be reduced accordingly.

Sample size

Due to the inhibitory nature of plant material, results obtained with the direct PCR protocol typically improve as the amount of material supplied as template decreases. To ensure consistent results, use a sampling tool (0.35–0.5 mm) to add a consistent, fixed amount of sample into each direct PCR.

With the crude sample PCR protocol, larger amounts of sample can be used, enabling the use of a scalpel for sampling purposes.

Direct or crude sample PCR

Crude sample PCR is generally preferred over direct PCR, for the following reasons:

- Precise control over sample size is not required
- Multiple PCRs can be performed from the same crude extract
- Crude extracts can be stored for 3–5 days at 2°C to 8°C, should any additional or repeat PCRs be required
- Reproducibility is higher than with direct PCR
- Much less optimization is required than for direct PCR
- Direct PCR does not work for all sample types.

$MgCl_2$ concentration

The KAPA Plant PCR Buffer contains $MgCl_2$ at a 1X concentration of 1.5 mM. This is typically sufficient for PCR from purified DNA, but direct and crude sample PCR may require additional $MgCl_2$. A final $MgCl_2$ concentration of 2 mM should be used as a first approach.

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Enzyme concentration

We recommend 1 U of KAPA3G Plant DNA Polymerase per 50 μL reaction with purified DNA. The direct and crude sample PCR protocol should be attempted with 1 U as a first approach, and increased in 1 U increments if necessary.

Primers

Primer quality is critical for successful PCR. To limit degradation, primers should be stored and diluted in 10 mM Tris-HCl (pH 8.0–8.5), and not in water. Furthermore, primers should be designed to have similar theoretical melting temperatures.

It may be useful to evaluate direct or crude sample PCR for a particular sample type with universal primers prior to attempting PCR with specific primers. This will determine if the sample type is amenable to direct or crude sample PCR.

GC-rich PCR

For GC-rich PCR (>65% GC content), reactions may be supplemented with 5% DMSO. Assays with extreme GC content can be difficult even when using purified DNA as template, and those assays may be more difficult to convert to direct or crude sample PCR.

Cycling protocol

KAPA3G Plant PCR Kits contain an engineered enzyme, and should not be used with cycling protocols typically used with wild-type *Taq*. An extension time of 30 sec/kb is sufficient for highly efficient amplification.

We recommend 35 cycles with purified DNA (~10 ng per reaction) as template, while direct and crude sample PCR typically require 45 and 40 cycles, respectively. The annealing temperature is one of the most critical parameters for successful PCR, regardless of the template used. The KAPA Plant PCR Buffer is designed to facilitate specific amplification over a wide annealing temperature range (~10°C), which limits the amount of annealing temperature optimization required. Start with an annealing temperature 2°C higher than the calculated melting temperature of the primers, and adjust up (to improve specificity) or down (to improve yield) as required.

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