Technical Data Sheet

KAPA3G Plant PCR Kit

KK7251 250 x 50 µL reactions	**KAPA3G Plant PCR Kit** Contains: - 1 x 250 U KAPA3G Plant DNA Polymerase (2.5 U/µL) - 1 x 6.25 mL KAPA Plant PCR Buffer (2X) - 1 x 125 µL KAPA Plant PCR Enhancer (100X) - 1 x 1.6 mL $MgCl_2$ solution (25 mM)
KK7252 500 x 50 µL reactions	**KAPA3G Plant PCR Kit** Contains: - 2 x 250 U KAPA3G Plant DNA Polymerase (2.5 U/µL) - 2 x 6.25 mL KAPA Plant PCR Buffer (2X) - 2 x 125 µL KAPA Plant PCR Enhancer (100X) - 1 x 1.6 mL $MgCl_2$ solution (25 mM)

1. Product Description

Amplification of plant-derived DNA is a challenging application due to the diversity of plant tissue types and the potent PCR inhibitors contained within the tissue. The KAPA3G Plant PCR Kit is optimized for the successful amplification of DNA from crude plant samples, DNA containing carry-over inhibitors from crude extraction methods, as well as purified DNA.

The KAPA3G Plant PCR Kit contains a novel DNA polymerase, engineered via a process of molecular evolution, for improved tolerance to common plant-derived PCR inhibitors such as polyphenolics and polysaccharides. The unique characteristics of the enzyme result in robust amplification across a wide range of plant sample types, amplicon lengths, and crude extraction methods. The kit contains 4 separate components: 1) KAPA Plant PCR Buffer (2X) is a ready-to-use cocktail containing all components except DNA polymerase, primers, and template. This buffer contains $MgCl_2$ at a 1X concentration of 1.5 mM; 2) KAPA3G Plant DNA Polymerase is a blend of an engineered *Taq*-based (Type A) DNA polymerase and a modified archaeal (Type B) DNA polymerase. This enzyme blend is combined with proprietary antibodies that inactivate the enzymes prior to the first denaturation step; 3) KAPA Plant PCR Enhancer is supplied as an optional additive to improve PCR performance for difficult samples and assays where $MgCl_2$ titration fails to improve results; 4) Additional $MgCl_2$ (25 mM) is supplied for assays that require $MgCl_2$ optimization.

DNA fragments generated with the KAPA3G Plant PCR Kits are A-tailed and suitable for use with TA cloning vectors.

Quick Notes

- KAPA3G Plant DNA Polymerase offers robust performance across a wide range of plant tissue types and inhibitors present either in crude plant samples or carried over during DNA extraction.
- Optimize PCR reaction conditions with purified DNA prior to attempting crude sample PCR (see Section 6).
- Excessive amounts of crude plant material in a PCR reaction is the major reason for failure. The Harris Uni-Core™ 0.5 mm or 0.35 mm sampling tools are recommended for sampling crude plant material, which allows consistent and small sample sizes (0.5 mm sampling tool: Sigma product no. Z708771).
- Use 1 unit of KAPA3G Plant DNA Polymerase (0.4 µL) per 50 µL PCR reaction. This may be increased to 2 - 5 units per 50 µL PCR reaction for difficult samples.
- KAPA Plant PCR Buffer (2X) contains $MgCl_2$ at a 1X concentration of 1.5 mM. A final $MgCl_2$ concentration of 1.5 mM is recommended for amplification from purified or extracted DNA, whereas 2.0 mM is recommended for crude samples.

2. Product Applications

The KAPA3G Plant PCR Kit is designed for the amplification of DNA fragments ≤5 kb in length from a range of plant samples including:

- Crude extractions of plant DNA containing carry-over inhibitors
- Directly from leaf discs, seed samples, and other plant tissue samples
- Samples containing significant concentrations of plant-derived compounds

3. Product Specifications

3.1 Shipping and Storage

KAPA3G Plant PCR Kits are shipped on dry ice or ice packs, depending on the country of destination. Upon receipt, store the entire kit at -20 °C in a constant-temperature freezer. KAPA3G Plant PCR Kits Kits may be stored at 4 °C for regular, short-term use (up to 1 week). When stored under these conditions and handled correctly, all kit components will retain full activity for at least six months from the date of receipt, or until the expiry date indicated on the kit.

3.2 Quality Control

All components contained with KAPA3G Plant PCR Kits are subjected to stringent quality control tests, are free of contaminating exo- and endonuclease activities and meet strict requirements with respect to DNA contamination.

3.2 Product Use Limitations

KAPA3G Plant PCR Kits are sold exclusively for research purposes and *in vitro* use. Neither the product, nor any individual component, was tested for use in diagnostic applications or for drug development, nor is it suitable for administration to humans or animals. Please refer to the MSDS, which is available upon request.

4. KAPA3G Plant PCR Kit protocol

A standard reaction setup is provided below:

	Final concentration	50 µL reaction[1]
PCR grade water up to 50 µL	-	As required
KAPA Plant PCR Buffer (2X) - contains $MgCl_2$[2] and dNTPs	1X	25 µL
Forward Primer (10 µM)	0.3 µM	1.5 µL
Reverse Primer (10 µM)	0.3 µM	1.5 µL
KAPA3G Plant DNA Polymerase (2.5 U/µL)	1 U/50 µL reaction[3]	0.4 µL
Template (0.5 mm or 0.35 mm diameter for crude sample, 1 – 10 ng for purified DNA)		As required
KAPA Plant PCR Enhancer (100X) OPTIONAL	As required (0 - 1X)	As required

[1]Reaction volumes smaller than 50 µL may be used for purified DNA or less inhibitory samples. However, a 50 µL reaction volume is recommended for difficult samples. Reaction volumes as low as 10 µL are possible with certain sample types.

[2]Crude sample PCR typically requires increased $MgCl_2$ concentration. Additional $MgCl_2$ (25 mM) is supplied for crude sample assays that require $MgCl_2$ optimization.

[3]Some sample types may require more than 1 unit of KAPA3G Plant DNA Polymerase per 50 µL reaction. Up to 5 units per 50 µL reaction may be used for especially recalcitrant sample types (see Section 6).

Important notes on reaction setup:

- Ensure that all components are completely thawed and mixed properly before use. Briefly centrifuge tubes before opening. Note that the KAPA Plant PCR Buffer is viscous; it is therefore recommended that the water is added first during reaction setup. This is followed by KAPA Plant PCR Buffer. To ensure that all the buffer has been added, flush the pipette tip by pipetting up and down a couple of times.

- The KAPA Plant PCR Buffer (2X) contains $MgCl_2$ at a 1X concentration of 1.5 mM. Separate $MgCl_2$ (25 mM) is supplied with the kit for use with assays that require additional optimization. A final concentration of 1.5 mM $MgCl_2$ is usually sufficient for extracted or purified DNA, whereas 2.0 mM $MgCl_2$ is recommended for crude samples.

- KAPA Plant PCR Enhancer (100X) is supplied with the kit and may be included in the reaction at 0.2 - 1X final concentration (0.1 - 0.5 µL/50 µL). KAPA Plant PCR Enhancer is recommended for difficult assays that do not improve after $MgCl_2$ and enzyme concentration have been optimized.

- Dilution buffer is recommended if:

  1. Crude samples directly in reaction do not lead to successful amplification.
  2. Multiple targets must be amplified from the same sample (especially when crude sample material is limiting).
  3. Storage of sample material is required in a convenient format for subsequent PCR.

  Dilution buffer setup [50 mM Tris-HCl (pH 8.0 - 8.5), 0.1 mM EDTA, 0.2X KAPA Plant PCR Enhancer]: Add a leaf disc of approximately 1.2 mm diameter (or similar-sized other sample) to 20 - 50 µL dilution buffer in a PCR tube, crush the sample with a pipette tip*, vortex, centrifuge, and use 0.25 - 5 µL of the supernatant for PCR. Samples may be stored in dilution buffer for a limited time at 4 °C or for longer periods at -20 °C. The stability of different plant samples will vary and should be tested by the user. For maximum storage life, it is strongly recommended that the sample is centrifuged and the supernatant transferred to a new tube for storage at -20 °C.

  *A useful tool for crushing plant samples can be made by briefly flaming a 100 µL pipette tip to seal it. The sealed end of the pipette tip will usually have a diameter suitable for crushing samples at the bottom of a PCR tube.

5. Cycling parameters

Standard cycling parameters are provided below:

Step	Temperature	Duration	Cycles
Initial denaturation	95 °C	3 - 10 min[1]	1
Denaturation	95 °C	20 sec[2]	**40[3]**
Annealing[4,5]	50 - 68 °C[4]	15 sec[4]	
Extension[6]	72 °C	30 sec/kb[6]	
Final extension	72 °C	30 sec/kb	1
Hold	4 - 10 °C	-	1

[1]For crude samples, use 10 min initial denaturation. For purified DNA, use 3 min initial denaturation. The initial denaturation time may be extended up to 20 min for some crude sample types, if needed.

[2]Use at least 20 sec cycling denaturation time for 50 µL reaction volumes. The denaturation time may be increased to 30 sec for complex targets.

[3]For crude samples, 40 PCR cycles are recommended as a starting point. Increase or decrease cycle number according to results obtained (see Section 6 for recommendations and guidelines for crude sample PCR).

[4]Use the average primer Tm + 2 °C to start. Performing an annealing temperature gradient PCR is recommended for further optimization. Start with 15 sec annealing time; the annealing time may be reduced to 10 sec if non-specific amplification is observed which cannot be improved by increasing the annealing temperature.

[5]For primers with optimal annealing temperatures of 68 °C or higher, a two-step cycling profile with annealing/extension at 68 – 72 °C may be used.

[6]Start with 30 sec/kb extension time. If non-specific products of greater length than the desired PCR product are problematic, reduce the extension time to 20 - 25 sec/kb.

6. Recommendations and guidelines for crude sample PCR

- **Annealing temperature:** Optimize with purified DNA. We recommend performing an annealing temperature gradient PCR (25 µL reaction volume). At minimum, the following annealing temperatures should be tested: the average primer Tm, Tm + 2 °C, Tm + 4 °C and Tm + 8 °C. If none of these annealing temperatures gives a satisfactory result, perform the gradient over a wider/finer range, up to 72 °C. If a primer set exhibits a wide Ta range, it is recommended that the highest Ta that results in acceptable yield is used.

- **Number of PCR cycles:** Note that the number of cycles required for successful crude sample PCR tends to be high (40 – 50 cycles are typical). This is mainly due to the very low copy number of starting template, the effect of PCR inhibitors, etc. Optimal cycle number for specific sample types/sizes must be determined empirically. High copy number chloroplast targets can be amplified using fewer PCR cycles (35 cycles are recommended as a first approach).

- **Sample size:** Due to the inhibitory nature of crude plant material, amplification from crude plant tissue tends to improve as the amount of sample in the reaction is reduced. The Harris Uni-Core™ sampling tools are recommended for maintaining consistently small amounts of plant tissue per reaction. The 0.5 mm diameter punch is recommended for most applications, but the 0.35 mm punch may improve results with more challenging sample types. It is recommended that leaf discs are placed directly into the liquid in the PCR tube, rather than adding the master mix to the leaf disc. Do not further crush or damage leaf discs after addition to the tube. For very small seeds, such as *Arabidopsis* or tobacco, first crush the seed against the bottom of the PCR tube and then add the master mix. For larger seeds, use the 0.5 mm Uni-Core™ sampling tool to remove as small a piece as is possible and then add it directly to the PCR tube containing the master mix. Include appropriate positive and negative control reactions.

- **Difficult sample types:** The KAPA3G Plant PCR Kit may be used for amplification directly from 0.5 mm diameter leaf discs from challenging species, such as grapevine, for which 2 units of enzyme per 50 µL reaction volume is recommended. For very difficult sample types, 45 - 50 PCR cycles are recommended. Furthermore, use of the 0.35 mm Harris Uni-Core may improve results with very difficult samples. Increasing the amount of enzyme (up to 5 units/50 µL reaction in extreme cases) may be necessary for some sample types. Particularly challenging sample types may require crushing the tissue in dilution buffer (see Section 4), or pre-treatment with a lysis reagent such as KAPA Express Extract.

7. Troubleshooting

The recommendations suggested below may first be tried individually, then in combination as appropriate:

Low yield or no amplification

- Increase the magnesium concentration and/or increase the number of PCR cycles
- For purified DNA, increase the amount of template
- For a crude tissue sample, decrease the amount of sample in the reaction/increase the reaction volume
- Use the dilution buffer protocol or KAPA Express Extract with the crude sample
- Increase the enzyme concentration
- Use KAPA Plant PCR Enhancer, BME or DTT in the reaction
- Decrease the annealing temperature
- Increase the extension time
- Check primer sequences

Non-specific amplification or smearing

- Increase the annealing temperature and/or decrease the annealing time
- Decrease the number of PCR cycles
- Decrease the primer concentration
- Decrease the magnesium concentration, if >1.5 mM was used
- Decrease the enzyme concentration, if >1 unit/50 µL reaction was used
- Decrease the extension time
- Decrease the amount of template
- Redesign the primers

8. Licensing

The purchase of this product conveys to the purchaser only the limited, non-transferable right to use the purchased quantity of the product for the purchaser's own research by the purchaser only under the following U.S. patent claims and foreign counterpart patent claims: U.S. Patent No. 5,436,149 (claims 6-16). No rights are granted to the purchaser to sell, modify for resale or otherwise transfer this product, either alone or as a component of another product, to any third party. Takara Bio reserves all other rights, and this product may not be used in any manner other than as provided herein. For information on obtaining a license to use this product for purposes other than research, please contact Takara Bio Inc., Seta 3-4-1, Otsu, Shiga 520-2193, Japan (Fax +81-77-543-9254).

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