Vita Plant Kit

FOR RESEARCH USE ONLY

Vita Plant Kit is a convenient 2X mastermix formulation of *VitaTaq® DNA polymerase* optimized for the challenging analysis of plant derived samples. The formulation is optimized to intercept plant-derived inhibitors and allows for amplification from purified DNA, crude extracts or plant material directly. All three options are described in this protocol. For especially difficult samples, 10X Plant Enhancer is included.

Product	Size	SKU
Vita Plant Kit	2 ml 2X Mastermix 500 µl 10X Plant Enhancer	PCCSKU1022
	5 ml 2X Mastermix 2x 500 µl 10X Plant Enhancer	PCCSKU1023

Storage

Store *Vita Plant Kit* at -20°C upon arrival and avoid repeated freeze and thaw cycles. Stored under these conditions, reagents can be used until the expiry date stated on the packaging.

Considerations before starting

The recommended reaction volume is 50 µl. This leaves enough spare volume to dilute samples and therefore present inhibitors. When working with purified DNA, it is possible to scale down once a successful protocol has been established. For PCR directly from plant material, reaction volumes <50 µl are not recommended unless sample size can be scaled down accordingly.

When using plant material directly, use a sampling tool to ensure that each reaction contains an equal amount of sample material. PCR results improve with a decreasing amount of sample material. When using crude extracts, larger amounts of sample can be used and processed.

Vita Plant Kit contains $MgCl_2$ at a final concentration of 2.5 mM adapted to the requirements of plant samples. It is not necessary to optimize $MgCl_2$ concentration any further.

If your application does not require the use of direct samples, crude extract is recommended. Extracts can be stored for 3-5 days at 4°C, used in multiple reactions, used to repeat PCRs and produce results with a higher reproducibility. Furthermore, direct PCR is not an option for some sample types (e.g. Eucalyptus spp.).

When designing an experiment with crude extract or plant material as template, it is advisable to first optimize PCR conditions with universal primers and then switch to specific primers once a method for the specific samples type is established.

For purified DNA, 35 cycles are recommended. However, crude extracts and direct PCR typically require more amplifications cycles (40-45). *Vita Plant Kit* is designed to facilitate primer annealing over a broad temperature range. Start with 2°C below the calculated melting temperature of your primers and adjust stepwise if necessary. If possible, run a gradient for the optimal annealing temperature. Higher annealing temperatures increase specificity, lower annealing temperatures increase yield.

PCR from purified DNA

Before using crude extract or plant material, PCR conditions should be optimized using purified DNA first.

Component	Volume	Final Concentration
2X Mastermix	25 µl	1X
Primer 1 (10 µM)	1 µl	0.2 µM
Primer 2 (10 µM)	1 µl	0.2 µM
Template DNA	X µl	1-50 ng / rxn
(optional) 10X Plant Enhancer	5 µl	1X
dH_2O	to 50 µl	

Cycling Protocol

Step	Cycles	Temperature	Time
Initial Denaturation	1	94°C	5 min
Amplification	35	94°C	30 sec
		50-65°C[1]	30 sec
		72°C	30 sec / kb
Final Extension	1	72°C	5 min

[1] depending on the T_m of your primers, start with T_m-2°C or optimize with a gradient.

Direct PCR

Direct PCR can be performed with seeds or leaves from many plant species. It is not recommended for plants that contain a high amount of inhibitors (e.g. Eucalyptus spp.). For direct PCR, the amount of sample material is critical for successful amplification; if too much plant material is added, amplification will most likely fail. Therefore, use of a sampling tool for consistent sample sizes (0.35 – 0.5 mm diameter) is advisable. If results from direct PCR are non-satisfactory or multiple analysis should be performed on one sample, PCR from crude extract may be the better choice.

Component	Volume	Final Concentration
2X Mastermix	25 µl	1X
Primer 1 (10 µM)	1 µl	0.2 µM
Primer 2 (10 µM)	1 µl	0.2 µM
Leaf / Seed Material	0.3-0.5 mm Ø	
(optional) TCEP[2]	X µl	0.5-5 mM
(optional) 10X Plant Enhancer	5 µl	1X
dH_2O	to 50 µl[3]	

[2] if results are poor or PCR has failed, addition of TCEP up to 5 mM may improve results.
[3] reaction volumes <50 µl are not recommended as sample material can hardly be scaled down accordingly

Cycling Protocol

Use the cycling protocol optimized with purified DNA before. Direct PCR will most likely require a higher number of cycles. Start from 40 and increase in 5 cycles increments if the yield is poor. Due to the presence of inhibitors, direct PCR may also require longer extension times. If 30 sec / kb do not yield a product, increase duration in 15 sec increments.

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PCR from crude extract

For PCR from crude extract, prepare the extract as stated below:

Extraction Buffer

50 mM Tris-HCl pH 8-8.5
0.1 mM EDTA
2% ß-mercaptoethanol (add freshly before use)
Optional: 1 mM TCEP

DTT (Dithiothreitol) at 10 mM may be used as an alternative to ß-mercaptoethanol. However, extracts with DTT are generally less stable and may decrease PCR yield.

Use TCEP only if extraction without does not work. You may need to evaluate extraction buffer with and without it to find the procedure that works best for your sample.

Extraction

This protocol describes a basic extraction procedure for plant material. It may be necessary to adapt it depending on your sample type.

- For leaf samples, cut ~ 5x5 mm pieces and place each into a microcentrifuge tube. For seeds, use a comparable amount of sample and crush with mortar and pestle if necessary.
- Add 100 µl of extraction buffer (enough to completely submerge your sample) and mash the sample gently into the buffer with a sterile pipette tip.
- Place on ice or heat at 95°C for 5 minutes. It is recommended to try both procedures to determine the best extraction method four your individual sample.

Stability of prepared extracts should be determined experimentally. Extracts prepared with ß-mercaptoethanol are typically stable for 3-5 days at 4°C.

Component	Volume	Final Concentration
2x Mastermix	25 µl	1X
Primer 1 (10 µM)	1 µl	0.2 µM
Primer 2 (10 µM)	1 µl	0.2 µM
Crude Extract	1.5 µl	
(optional) TCEP2	X µl	0.5-5 mM
(optional) 10X Plant Enhancer	5 µl	1X
dH_2O	to 50 µl	

Cycling Protocol

Use the cycling protocol optimized with purified DNA before. Crude extract PCR will most likely require a higher number of cycles. Start from 40 and increase in 5 cycles increments if the yield is poor. Due to the presence of inhibitors, crude extract PCR may require longer extension times. If 30 sec / kb do not yield a product, increase duration in 15 sec increments.