



## Product information

---

**Product Name:** **Direct Tissue PCR Kit**

**Catalog No:** VN440DK, VN441DK, VN442DK, VN440DKR, VN441DKR, VN442DKR, VN440DKGC, VN441DKGC, VN442DKGC, VN440DKRGC, VN441DKRGC, VN442DKRGC

**Packing Size:** 500 x 25 µl rxns, 250 x 25 µl rxns and 100 x 25 µl rxns

**Shipping Condition:** Ambient temperature or ice pack

**Storage Condition:** -20°C

**Shelf life:** At least 2 year if stored at -20°C or at least 1 week if stored at 4°C for 2X Direct Tissue PCR Mix. The polymerase should always be stored at -20°C and the shelf life will be at least three years from date of receipt.

**Product Description:**

**Direct Tissue PCR Kit** contains all the reagents needed for quick amplification of genomic DNA from human and animal tissue. This kit can be also applied to direct amplification of exogenous target genes, such as virus, bacteria and parasites from tissue. The kit is optimized with our unique inhibitor-resistant DNA polymerase for best performance. In most case, you can directly add sample into PCR master mix without pre-lysis.

There is the **choice of an inert green dye** in the mix, used for direct gel loading. The PCR product can be directly loaded on agarose gel without addition of loading dye for electrophoresis.

GC version works for GC-rich and complex target.

**List of Components:**

- Direct Tissue PCR Polymerase: 2 x 125 µl (500 rxns), 1 x 125 µl (250 rxns) and 1 x 50 µl (100 rxns)
- 2X Direct Tissue PCR Mix: 5 x 1.25 ml (500 rxns), 3 x 1.05 ml (250 rxns) and 1x 1.25 ml (100 rxns)
- Lysis Buffer: 5 x 1.5 ml (500 rxns), 4 x 1.5 ml (250 rxns) and 2 x 1.5 ml (100 rxns)

**Protocol:**

**Option A:** Directly add 0.1-0.2 mm of tissue to PCR. **Please always try this option first.**

**Option B:** Mix 0.1-0.2 mm of tissue with 20 µl of Lysis buffer in 0.2 ml PCR tube, put it in PCR cyclor at 97°C for 15 minutes and then cool down on ice, centrifuge at 12,000 rpm for 5 min. The supernatant containing crude DNA extracts is ready for PCR.

## PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
2X Direct Tissue PCR Mix	12.5 µl	1X
Direct Tissue PCR Pols†	0.25-0.5 µl	
Left Primer	Variable	0.2-0.5 µM
Right Primer	Variable	0.2-0.5 µM
Tissue / Crude Extract*	0.1-0.2 mm / 5 µl	20%
De-ionized Distilled H <sub>2</sub> O	Adjust final volume to 25 µl	-

† An enzyme titration from 0.125 to 1.0 µl can be performed to find the optimal concentration.

\*Please centrifuge the samples at 12,000 rpm for 2 minutes after PCR to pellet cell debris and then load 5-10 µl of supernatant to run a gel. You may see smear bands if the tissue in the PCR is too big to generate too much denatured protein in the reactions. So, we recommend you to use as small of tissue as possible in the PCR if you use first Option even though our Taq mutant is able to tolerate higher concentration of the inhibitors.

## Typical Cycling Parameters

Initial denaturation	95°C	10-15 min (for Option A) 2-5 min (for Option B)
Then followed by 30-45 cycles		
Denaturation	94°C	20 to 60 sec
Annealing	50°C to 68°C	20 to 60 sec
Extension	70°C	2 min / 1kb target
Final Extension	70°C	5 min
Hold	4°C	

## Troubleshooting guide

No PCR products	Check your PCR setting to see if you miss some components in the PCR master mix. Try gradient annealing temperature to find optimal one for your target.
Low yield of product	Make sure to initially denature samples for 10-15 min to release DNA if you use Option A. Increase PCR cycles. Enzyme titration. Increase extension time. Try gradient annealing temperature. Redesign primers.
Non-specific products are observed	Try gradient annealing temperature to find optimal one for your target. Check GC content of the target. If it is more than 65%, you may need to use Direct Tissue PCR GC Kit. Check your primers or redesign them if necessary.

**Warning:** if the reagent is spilled to your skin or eyes, please wash with large amount of clean water and call emergency care if necessary

**Note:** *This product is for R&D use only*