Product information

Product Name: Direct Cell PCR Kit

Catalog No: VN430DK, VN431DK, VN432DK, VN430DKR, VN431DKR, VN432DKR, VN430DKGC, VN431DKGC, VN432DKGC, VN430DKRGC, VN431DKRGC, VN432DKRGC

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^{0}C

Shelf life: At least 2 year if stored at -20^{0}C or at least 1 week if stored at 4^{0}C for 2X Direct Cell PCR Mix. The polymerase should always be stored at -20^{0}C and the shelf life will be at least three years from date of receipt.

Product Description:

Direct Cell PCR Kit contains all the reagents needed for quick amplification of genomic DNA from cell culture. This kit can be also applied to direct amplification of exogenous target genes, such as virus and parasites from cell culture. The kit is optimized with our unique inhibitor-resistant DNA polymerase and buffer 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 Cell PCR Polymerase: 2 x 125 µl (500 rxns), 1 x 125 µl (250 rxns) and 1 x 50 µl (100 rxns)
- 2X Direct Cell 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 0.25-5 µl of cell culture to PCR. Please always try this option first.

Option B: Mix 5 µl of cell culture with 20 µl of Lysis buffer in 0.2 ml PCR tube, put it in PCR cycler at 95^{0}C for 15 minutes and then cool down on ice, centrifuge at 12,000 rpm for 5 min. The supernatant containing crude DNA extracts are ready for PCR.

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PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
2X Direct Cell PCR Mix	12.5 µl	1X
Direct Cell PCR Pols†	0.5 µl	
Left Primer	Variable	0.2-0.5 µM
Right Primer	Variable	0.2-0.5 µM
Cell Suspension / Supernatant / Crude Extract*	≤ 5µl / ≤5 µl <10 µl	≤ 20% < 40%
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

† An enzyme titration from 0.25 to 1.0 µl can be performed depending on cell type and amount.

*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 not have enough supernatant for electrophoresis if the cell concentration in the PCR is too high. So, we recommend you to use up to 20% of cells / cell suspension in the PCR even though our Taq mutant is able to tolerate higher.

Typical Cycling Parameters

Initial denaturation	95^0C	6-10 min (for Option A) 2-5 min (for Option B)
Then followed by 30-45 cycles		
Denaturation	94^0C	20 to 40 sec
Annealing	50^0C to 68^0C	30 to 60 sec
Extension	70^0C	2 min / 1kb target
Final Extension	70^0C	5 min
Hold	4^0C	

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 6-10 min to release DNA if you use protocol 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 annealing temperature for your target. Check GC content of the target. If it is more than 65%, you may need to use our Direct Cell 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

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