For life science research only.
Not for use in diagnostic procedures.

PCR ELISA (DIG-Labeling)

For direct labeling of PCR products with digoxigenin.

Cat. No. 11 636 120 910

Version 11

Content version: April 2019

Store at −15 to −25°C

1. Kit contents

Vial/ Cap	Label	Contents/function
1 violet	PCR DIG labeling mix	• 2 x 250 µl, [2 mM dATP, dCTP, dGTP, 1.9 mM dTTP and 0.1 mM DIG-dUTP] • nucleotide mix for PCR reaction
2 yellow	PCR reaction buffer (10 × conc.) without $MgCl_2$	• 1.1 ml, [100 mM Tris-HCl, 500 mM KCl, pH 8.3 (+20°C)]. • buffer to optimize the PCR reaction
3 blue	$MgCl_2$-stock solution	• 1.1 ml, [25 mM $MgCl_2$] • for $MgCl_2$ titration during the PCR reaction
4 green	PCR buffer (10 × conc.)	• 1.1 ml, [100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3(+20° C)] • buffer for PCR labeling reaction
5	Taq DNA Polymerase	• 5 U/µl • 125 units [20 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM EDTA, Nonidet[1)] P40, 0.5% (v/v), Tween[2)] 20, 50% (v/v), glycerol, 0.5% (v/v), pH 8 (+4°C)]
6	Control PCR Primer Mix	• 50 µl • 125 pmol of each primer • mixture of primers (two oligonucleotides) for the control reaction, specific for the human tPA gene
7	Human control DNA	• 50 µl, [3 ng/µl] • template DNA for control reaction
8	Water, sterile, PCR grade	4× 1.1 ml sterile double distilled water.

2. Product overview

Test principle
Taq DNA Polymerase incorporates digoxigenin-11-dUTP (DIG) into target DNA in 25–35 amplification cycles.

Application
The kit is used for nonradioactive labeling of DNA by PCR. For detection and quantification of the PCR product, we recommend to use the PCR ELISA, DIG Detection Kit*.

Assay time/ Hands on time
Labeling by PCR will take 3.5–4 h including 10–40 min hands on time.

Number of tests
The kit is designed for 50 polymerase chain reactions incorporating DIG dUTP.

Quality control
DIG-labeling is function tested with the described control reactions. All reagents are free of contaminants.

Storage and stability
Stable at −15 to −25° C until the control date printed on the label.

Once thawed keep vial 7, human control DNA, at +2 to +8°C.

3. Procedure

Mg^{2+} concentratation
In most cases a concentration of 1.5 mM Mg^{2+} will produce satisfactory PCR results. Generally, higher Mg^{2+} concentrations increase the PCR yield, but decrease the specificity of the reaction (increasing the incidence of primer dimers). Lower Mg^{+2} concentrations increase the specificity, but decrease the yield.

Prior to the procedure
All reagents except *Taq* polymerase must be thawed, mixed thoroughly (vortex), and centrifuged briefly before use.

Procedure
We recommend strongly to run a negative control to check for cross contamination from the reagents. For this control perform an identical assay, omitting template DNA.

Please refer to the following table to run the assay.

Step 1a

Action

For the control reaction, add the following components in a 1.5 ml reaction tube on ice in the same order as described below:

Component	Vol.	Final conc.
Sterile water (vial 8)	55.5 µl	
PCR reaction buffer (10× conc.), without $MgCl_2$ (vial 2)	10 µl	1×
$MgCl_2$-stock solution (vial 3)	4 µl	1.0 mM
PCR DIG labeling mix (vial 1)	10 µl	200 µM
Control primers (vial 6)	10 µl	250 nM
Taq DNA polymerase (vial 5)	0.5 µl	2.5 U
Human control DNA (vial 7)	10 µl	30 ng
Total volume	100 µl	

Step 1b

Action

For the sample reaction, add the following components in a 1.5 ml reaction tube on ice in the same order as described below:

Component	Vol.	Final conc.
Sterile water (vial 8)	variable	
PCR reaction buffer (10× conc.), without $MgCl_2$ (vial 2)	10 µl	1×
$MgCl_2$-stock solution (vial 3)	2–10 µl	0.5−2.5 mM
PCR DIG labeling mix (vial 1)	10 µl	200 µM
Target specific primers	variable	250 nM
Taq DNA polymerase (vial 5)	0.5 µl	2.5 U
Sample DNA	variable	1 fg−10 ng
Total volume	**100 µl**	

Step 2

Action

Mix the reagents thoroughly and centrifuge briefly.

Overlay the reaction carefully with 100 µl mineral oil to reduce evaporation.

Note: Mineral oil can be omitted if you are using a PCR cycler that does not need an oil-overlay (according to the recommendations of the manufacturer).

Step 3

Action

Place samples in a thermal cycler and start appropriate cycling program. The cycling program for the control reaction supplied in this kit is given below:

Number of cycles	Reaction	Temperature	Time period
1	initial denaturation	+95°C	5 min
30	denaturation hybridization elongation	+95°C +60°C +72°C	45 sec 1 min 2 min
	final elongation	+72°C	up to 10 min

This program can be also used for your individual template/primer pair, however for optimal results, adjust cycling parameters to your specific application.

Storage of the PCR product

Please refer to the following table, if you wish to store your PCR product.

Storage	Temperature
Short term	+2 to +8°C
Long term	−15 to −25°C

4. Supplementary Information

Changes to Previous Version

Editorial changes.

Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
Numbered Instructions labeled ①, ②,etc.	Steps in a process that usually occur in the order listed
Numbered Instructions labeled ❶, ❷,etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this document the following symbols are used to highlight important information:

Symbol	Description
🔍	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Ordering Information/

Product	Pack size	Cat. No.
PCR ELISA (DIG Detection)	192 reactions	11 636 111 910
PCR ELISA (DIG Detection), 5-Pack	480 reactions	11 965 409 910
PCR Optimization Kit	1 kit	11 636 138 001
PCR Nucleotide Mix	100 reactions	11 581 295 001
Taq DNA Polymerase, 5 U/µl	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001

Trademarks

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Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

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