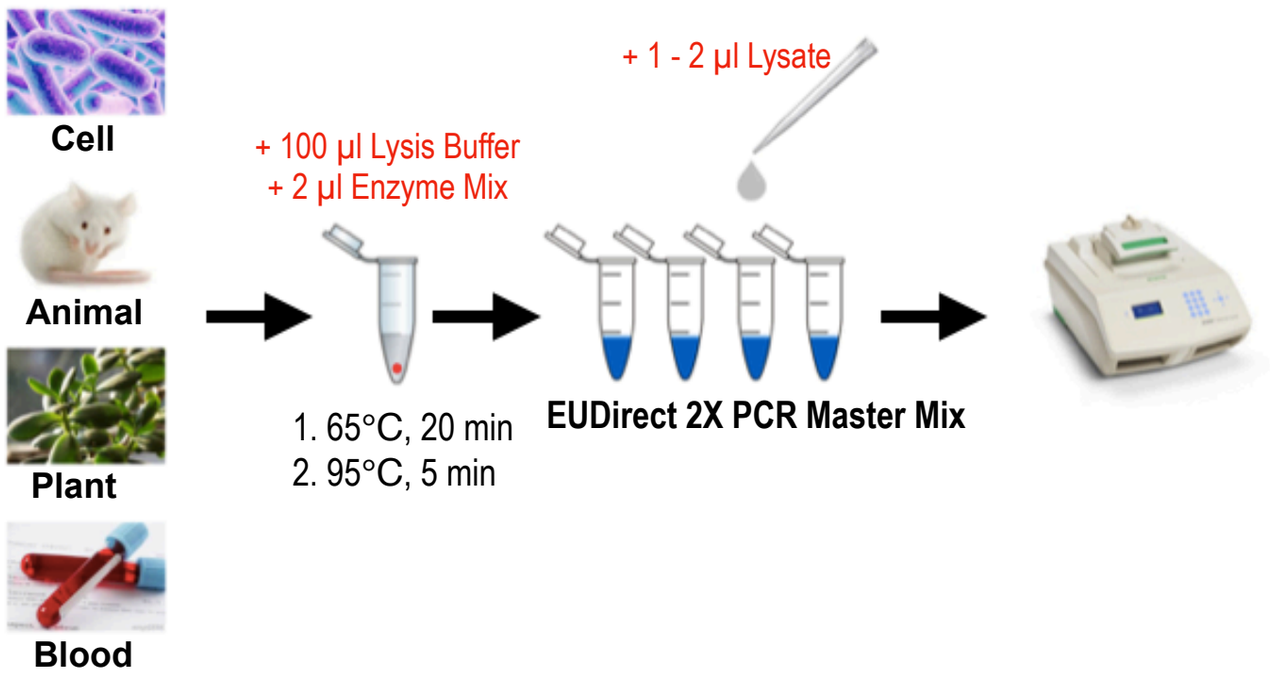
EUDirect PCR Kit

Components

Catalog	Description	Quantity	Storage
EUD12S	EUDirect 2X PCR Master Mix	4 X 1.5 mL	-20°C
EUD12LS	EUDirect Lysis Buffer	25 mL	RT
EUD12E	EUDirect Enzyme Mix	0.5 mL	-20°C
EUD12EC	Eukaryote Control Primer Mix	100 µl	-20°C
EUD12PC	Prokaryote Control Primer Mix	100 µl	-20°C

Product Description

EU-Direct PCR Master Mix facilitates PCR amplification directly from small amount of samples without DNA extraction and purification. It allows direct PCR amplification from a variety of samples including whole blood, blood collected on card, saliva, bacteria, mouse tail, tissue, cultured cells or plant.



Lysis protocol

1. Add suggested amount of starting material to 1.5 mL eppendorf tube
   - *Bacteria/mammalian cells*: 10 - 20 µl of cells
   - *Mouse and other animals*: 0.1 - 0.2 cm sample (using a puncher or scalpel)
   - *Plant and seed*: 0.1 - 0.2 cm sample (using a puncher or scalpel)
   - *Blood*: 2 - 10 µl of whole blood
2. Add 100 µl of Lysis Buffer and 2 µl of Enzyme Mix to each tube, vortex briefly
3. Incubate the tube at 65 ℃ for 20 minutes, then transfer to 95 ℃ for 5 minutes
4. Use 1 - 2 µl of lysates for PCR reactions

PCR Setup

	50 µl reaction	Final
EUDirect 2X PCR Master Mix	25 µl	1X
10 µM primer A	2.5 µl	500 nM
10 µM primer B	2.5 µl	500 nM
Template DNA (lysates)	1 - 2 µl	
Nuclease-free water	to 50 µl	

Cycling Condition

	Temperature	Time	Cycle
Initial Denaturation	98 °C	3 min	1
Denaturation	98 °C	10 sec	30 - 35
Annealing	52 - 64 °C	30 sec	
Extension	72 °C	30 - 60 sec/kb	
Final Extension	72 °C	10 min	1
Hold	4 °C		

- Recommended annealing temperature 56°C - 60°C as the starting point.

http://www.eu-bio.com
Technical support: support@eu-bio.com

EUDirect PCR Kit

Universal control primer mix

The kit includes two primer mixes to use as the positive control:

- *Eukaryote Control Primer Mix (100 µl - enough to run 20 reactions)*: Contains primers designed to amplify the specific region (target size 150 bps) of eukaryotic 18s DNA. This should be used when performing direct PCR with blood, mammalian cells, mouse, animal or plant.
- *Prokaryote Control Primer Mix (100 µl - enough to run 20 reactions)*: Contains primers designed to amplify the specific region (target size 700 bps) of prokaryotic 16s DNA. This should be used when performing direct PCR with bacteria cells.

PCR setup for control reaction

	50 µl reaction	Final
EUDirect 2X PCR Master Mix	25 µl	1X
Eukaryote Control Primer Mix (or Prokaryote Control Primer Mix)	5 µl	500 nM
Template DNA (lysates)	1 - 2 µl	
Nuclease-free water	to 50 µl	

Cycling Condition for control reaction

	Temperature	Time	Cycle
Initial Denaturation	98 °C	3 min	1
Denaturation	98 °C	10 sec	30 - 35
Annealing	58 °C	30 sec	
Extension	72 °C	30 sec	
Final Extension	72 °C	5 min	1
Hold	4 °C		

Troubleshooting

No product or low yield
Perform PCR with Control Primer Mix to determine which step is not working
Make sure that the heating step (95°C, 5min) is performed correctly
Perform a temperature gradient PCR
Increase the number of cycles or denaturation time
Check the purify and concentration of the primers
Add 1-5% DMSO (for GC amplicons)
Make sure not using too much sample in the Lysis step. Use water to dilute the lysate if it is necessary.
Titrate the amount of lysate used in PCR reaction (Recommendation is 1-2 µl. But can add up to 10% of the total PCR reaction volume.
Non-specific products
Make sure that the extension time was not too long (Recommendation is 30-60s/kb)
Increase annealing temperature or perform a temperature gradient PCR
Reduce the total number of cycles
Decrease primer concentration
Design new primers

Shipping and Storage

We ship at room temperature to reduce the shipping cost. The product is stable at 37°C for several weeks and can be stored at 4°C for several months. For long-term storage, please keep it at -20°C.

http://www.eu-bio.com
Technical support: support@eu-bio.com