# BlooDireX PCR™ System

Cat. No.: SM211-0100 Size: 100 reactions
Cat. No.: SM211-0010 Size: 10 reactions
Store at -20°C

## Description

The BlooDireX PCR™ System is designed for amplification of DNA from whole blood. It contains BlooDireX PCR and Novel Juice, suitable for PCR from blood sample directly. BlooDireX PCR™ is performed PCR directly from sample with no prior DNA extraction or sample preparation. For blood, stored at 4 °C or frozen, and preserved with EDTA, citrate or heparin are all suitable for this master mix. BlooDireX PCR™ is a premixed ready- to-use solution containing reaction buffers, dNTPs, loading dye, glycerol, PCR enhancers, and hot- start DNA polymerase. The hot-start taq prevents non-specific amplification due to mispriming and/or formation of primer dimers before thermal cycling, and it also exhibits 2X faster elongation rate than conventional Taq. The modified hot-start DNA polymerase is resistant to most PCR inhibitors and retains its activity in the PCR reaction. BlooDireX PCR™ is supplied at the 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of primer and template solutions. Reagents are provided with the sufficient amplification reactions of 20 µl each. Novel Juice is a non-mutagenic fluorescent reagent that produces instant visualization of DNA bands upon blue light (e.g. BLooK, BK001) or UV illumination of agarose gels. Supplied in 6X DNA Loading Buffer, Novel Juice is used to prepare DNA markers or samples for loading on agarose or polyacrylamide gels. Novel Juice is the most sensitive staining reagent available for detecting the double-stranded DNA (dsDNA). It contains three tracking dyes (bromophenol blue, xylene cyanol FF, and orange G) for visually tracking the DNA migration during the electrophoresis process. It is ideal for the environment requiring a safe, non-hazardous alternative to Ethidium Bromide (EtBr). Approximate fluorescence excitation / emission: 300, 495 / 537 nm, bound to nucleic acid.

## Features

- Sample is added directly to PCR reaction, therefore there is no need for time-consuming and expensive DNA purification steps.
- Master Mix format with premixed gel loading dye minimizes possibility of cross-contamination, reduces sample handling time and allows directly loading to gel.
- Amplify fragment size: up to 5 kilo base.
- High speed: 15-30 seconds/ kilo base.

## Applications

- Genotyping.
- Gene knockout analysis.
- Transgenic detection.

## Kit Contents

| Contents | SM211-0100 | SM211-0010 |
|---|---|---|
| BlooDireX PCR™ | 1000 µl | 100 µl |
| Novel Juice | 1000 µl | 50 µl |

## Quality Control

The quality of the BlooDireX PCR™ is tested on a lot-to-lot basis to ensure consistent product quality.

## Required Materials

Blood samples
PCR tubes
PCR thermal cycler
Electrophoresis equipment

## BlooDireX PCR™ System Protocol

The following table is recommended input size of materials:

| Materials | Input size |
|---|---|
| Blood | 1-10 % in the PCR Reaction |

1. For each 20 µl reaction, assemble the following components in a 0.2 ml PCR tube on ice before the experiment:

| Component | Volume (µl) | Final Concentration |
|---|---|---|
| BlooDireX PCR™ | 10 | 1X |
| Forward primer, 5-10 µM | Variable | 0.1-0.2 µM |
| Reverse primer, 5-10 µM | Variable | 0.1-0.2 µM |
| Raw material | Variable | - |
| Add dd$H_2O$ to | 20 | |

2. Mix gently. If necessary, centrifuge briefly. Cap the tube and place it in the thermal cycler.
3. To process in the thermal cycler for 25-35 cycles as follows:

| Process | Temperature (°C) | Time | Cycles |
|---|---|---|---|
| Initial Denaturation | 95 | 10 minutes | 1 |
| Denaturation | 95 | 30 seconds | 25-35 |
| Annealing | 45-65* | 30 seconds | |
| Extension | 72 | 30 seconds | |
| Final extension | 72 | 5 minutes | 1 |
| Hold | 4 | ∞ | 1 |

*Note: It may be necessary to optimize the system for individual primers, template, and thermal cycler.

4. After the PCR reaction, place the PCR product tubes on ice for the following analysis steps.
   *Note: We recommended vortex the Novel Juice for 10 seconds prior to use.
   Dilute 1 part Novel Juice with 5 parts PCR product and mix.
   *Note: Novel Juice must be added to DNA markers in order to visualize the ladder bands simultaneously with the sample after electrophoresis.
5. Start electrophoresis.
6. After the electrophoresis, remove gel and place on UV or a visible-light transilluminator to immediately visualize bands.
7. Gels can be post-stained with EtBr if desired.

## Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when PCR with the reagent:

| Problem | Cause | Solution |
|---|---|---|
| Low yield of PCR products | Incomplete concentration of start materials | Use the appropriate method for the DNA preparation based on the amount of the starting materials. |
| There is no PCR band | Samples contain impurities that include PCR inhibitors | Try reducing the amount of starting material, thereby reducing the concentration of PCR inhibitors. |
| Non-specific, multiple bands, or a smear are observed | Primers are non-specific | Optimized the PCR condition. |
| | DNase contaminant | Maintain a sterile work environment to avoid contamination from DNase. |

## Related Ordering Information

| Cat. No. | Description | Size |
|---|---|---|
| SA001-0500 | AGAROSE Tablets | 0.5g x 100 tablets |
| SD010-R600 | 1Kb DNA Ladder RTU | 600 µl |
| BK001 | BLooK LED Transilluminator | Set |
| BK002 | *p*BLooK LED transilluminator | Set |
| BK003 | *µ*BLooK LED Transilluminator | Set |