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# *EMD* gene amplification kit for direct sequencing (STRIPS)

## MBK0031
## 96 REACTIONS /12 DNA samples

*EMD coding regions amplification for direct sequencing application*

## FOR RESEARCH USE ONLY

**NAME AND INTENDED USE**

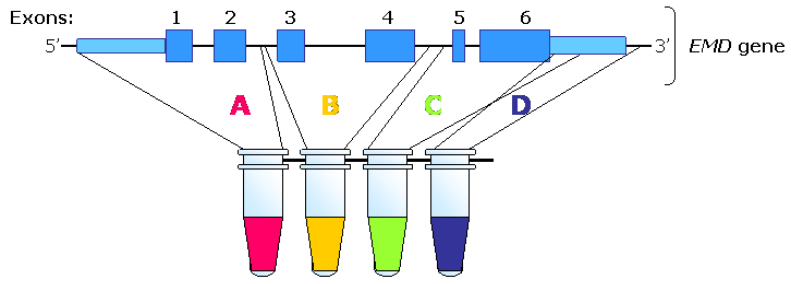
Ready-to-use strips for the simultaneous amplification of human EMD gene exons with modified primers: amplicons can be used for direct sequencing with M13 universal primers.

**INTRODUCTION**

Emerin is a serine-rich inner nuclear membrane protein and a member of the nuclear lamina-associated protein family; it is expressed in cardiac, skeletal, and smooth muscle.
Multiple functions for emerin have been proposed, including the stabilization of the nuclear membrane during muscle contraction and organizational maintenance of the nuclear membrane during cell division.
Emerin is encoded by the gene *EMD*, which is composed of six exons and is located at Xq28.
**X-linked recessive Emery-Dreifuss muscular dystrophy (EDMD)** arises as consequence of mutations in *EMD* and is characterized by early contractures of the Achilles tendons, elbows, and post-cervical muscles with humero-peroneal weakness and muscle wasting. Affected individuals also have cardiac conduction defects and may have a generalized cardiomyopathy.

**PRINCIPLE OF THE ASSAY**

The kit is composed of 6 strips. Each strip consists of 8 (4+4) tubes and allows the amplification of two DNA samples. Each vial contains all reagents and primers necessary for the amplification of a specific EMD gene region, as illustrated in the figure and table below.




| PCR mix | A | B | C | D |
|---|---|---|---|---|
| **Sequence amplified** | 5'UTR - **Exo 1** – Intro 1- **Exo 2** | **Exo 3** - Intro 3 – **Exo 4** | **Exo 5**-Intro 5- **Exo 6** | 3'UTR |
| **Amplicon length** | 590 bp | 559 bp | 582 bp | 585 bp |

PCR primers are composed of a specific EMD target sequence with flanking priming sites for M13 Forward or Reverse primers.


Their special composition allows the sequencing of all EMD coding regions using only two universal primers (M13 forward and M13 reverse). As consequence, only two sequencing reaction mixes are sufficient to obtain the entire coding sequence of the gene, avoiding the use of several sequencing primers.

**KIT CONTENTS**

***EMD* gene amplification strip:** n°6 strips. Each vial contains 8.8µl PCR mix.
**Hot-Rescue DNA Polymerase (5U/µl):** 30 µl (150U)

**OTHER SUPPLIES REQUIRED**

- Disposable latex gloves
- Precision pipettes
- Sterile pipette tips
- Sterile 1.5 ml vial
- Tabletop centrifuge with microplate adapters
- Optional: ExoSAP-IT (USB Corporation)

**STORAGE**

Store the kit at -20°C.

**GENERAL PRECAUTIONS FOR PCR**

The operator should always pay attention to:

- use pipette tips with filter;
- store positive material (specimens, controls and amplicons) separately from all other reagents and, if possible, add it to the reaction mix in a facility separated space;
- thaw all components and samples at room temperature before starting an assay;
- work on ice.

## PROTOCOL

### 1.1 DNA ISOLATION

Various protocols or kits can be used to extract genomic DNA.

- Carry out the DNA isolation according to the chosen procedure making sure that the extracted DNA is free from PCR inhibitors.
- Determine the concentration of the isolated DNA by standard methods.
- If necessary, dilute the genomic DNA sample with sterile deionized water or 10 mM Tris pH 8.0.
- Store the diluted DNA at -20°C avoiding repeated freeze/thaw cycles.

### 1.2 PCR SET UP

- Thaw the necessary number of PCR strip and centrifuge briefly.

  **NOTE:** calculate 4 vials for each DNA sample to analyze. Every strip provided is sufficient for two DNA samples.

- In a separated sterile 1.5 ml vial, mix the DNA sample with the Hot-Rescue DNA Polymerase enzyme provided, following the pipetting scheme below:

| FOR 4 REACTIONS (1 DNA SAMPLE) | VOLUME |
|---|---|
| **Genomic DNA Sample 50 ng/reaction** | 5.0µl |
| **Hot-Rescue DNA Polymerase 5U/ µl** | 1.0µl |

**NOTE:** for reaction blank use distilled water instead of DNA.

- Mix gently and centrifuge briefly.
- Aliquot 1.2 µl of the mix into each of the 4 vials of the strip.
- Centrifuge briefly.

### 1.3 PCR RUN

- Program the PCR thermal cycler with the following parameters:

| | |
|---|---|
| **1 cycle** | 95°C for 15 min |
| **35 cycles** | 95°C for 30 sec |
| | 70°C for 30 sec |
| | 72°C for 60 sec |
| **1 cycle** | 72°C for 7 min |
| | cool down to 4°C |

- Set the reaction volume to **10 µl.**
- Perform the PCR run.

When the run is completed, proceed immediately to the next step or store the reaction at +4°C overnight or at –20°C for longer time.

### 1.4 AMPLICON PURIFICATION

Before proceeding with the sequencing reaction, excess of primers and nucleotides must be removed by a purification procedure.

We recommend the use of ExoSAP-IT (not provided), following this procedure:
- centrifuge the PCR vials briefly;
- in each amplified reaction add 4 µl of ExoSAP-IT (USB Corporation);
- mix and centrifuge briefly;
- incubate the vials in the PCR thermal cycler executing the program below:

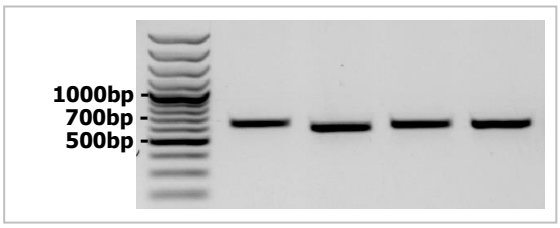
| | |
|---|---|
| **Enzyme incubation** | 37°C for 15 min |
| **Enzyme inactivation** | 80°C for 15 min |
| | cool down to 4°C |

- continue the protocol or store the amplicons at -20°C.

Alternative purification methods can be used but must be optimized by the operator.

### 1.5 AGAROSE GEL ELECTROPHORESIS

Before proceeding with the sequencing reaction, the quality and the final yield of the amplified DNA can be estimated by an agarose gel electrophoretic analysis.
- Mix 2µl of the purified PCR reaction with 8 µl of sterile water and 5 µl of DNA loading buffer.
- Separate the DNA on a 1.5% agarose gel containing ethidium bromide, in the presence of a DNA standard specific for the low range (100-1000 bp).




Lane 1: Generuler 100bp plus DNA Ladder (Fermentas)

Lanes 2-5: Purified amplicons A-D

### 1.6 DNA SEQUENCING

Each amplicon should be sequenced by M13 Forward and M13 Reverse primer (not provided).
M13 Forward primer allows the sequencing of 5′ amplicon region while M13 Reverse primer to obtain the sequence of the 3′ amplicon end.

M13 Forward primer (M13-21): 5′-**TGT AAA ACG ACG GCC AGT**-3′

M13 Reverse primer: 5′-**CAG GAA ACA GCT ATG ACC**-3′

**REFERENCES** Available on library section: **http://www.diatheva.com/library.htm**