CURRENT

Category: General SOP for preparation and performing horizontal gel electrophoresis in Genetics laboratory.
Title: Performing the horizontal gel electrophoresis for extracted DNA samples and PCR products in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Post-PCR method -03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Category: General SOP for preparation and performing horizontal gel electrophoresis in Genetics laboratory.

Title: Preparation and performing the horizontal gel electrophoresis for extracted DNA samples and PCR product in the Department of Clinical Pharmacology, K.E.M Hospital, Parel, Mumbai.

SOP No.: Post-PCR method -03 **Total pages:** 06

Date first effective: 1st January 2025 **Review date:** 31st December 2025

Version: 07

Author: Dr.Sheetal Kudtarkar
Project Scientist

Signature with date: 30/12/24

Reviewer 1: Dr. Bhaskar Krishnamurthy
Assistant Professor

Signature with date: 30/DEC/2024

Dr. Bhaskar Krishnamurthy
Assistant Professor,
Department of Clinical Pharmacology,
Seth GSMC and KEMH,Mumbai -400 012.

Reviewer 2: Dr. Mahesh Belhekar
Associate Professor

Dr. Mahesh N. Belhekar
Associate Professor
Department of Clinical Pharmacology
New MS Building, First Floor,
Seth GS Medical College and KEM Hospital
Acharya Donde Marg, Parel,
Mumbai - 400 012. India

Signature with date: 30/Dec/2024

Approved by: Dr. Nithya Gogaty
Professor & Head

Signature with date: 30/12/24

Dr. Nithya Gogtay
Professor & Head
Department of Clinical Pharmacology
1st Floor, MS Building,
Seth GS Medical College & KEM Hospital,
Parel, Mumbai - 400 012.

Category: General SOP for preparation and performing horizontal gel electrophoresis in Genetics laboratory.
Title: Performing the horizontal gel electrophoresis for extracted DNA samples and PCR products in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Post-PCR method -03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Table of contents

Category: General SOP for preparation and performing horizontal gel electrophoresis in Genetics laboratory.
Title: Performing the horizontal gel electrophoresis for extracted DNA samples and PCR products in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Post-PCR method -03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the method for preparation and performing the gel electrophoresis for extracted DNA from blood samples in Department of Clinical Pharmacology (DCP), Seth GS Medical College and KEM Hospital, Mumbai.

Scope: This SOP is limited for preparation and performing the horizontal gel electrophoresis for the extracted DNA from blood samples and for Polymerase Chain Reaction (PCR) Product.

Responsibilities:

Primary Responsibility: Divya Bhere
Lab Technician
30/Dec/2024

Secondary Responsibility: Dr.Sheetal Kudtarkar
Project Scientist
30/12/24

References: Molecular Cloning: A laboratory manual 3rd Edition (JF Sambrook & DW Russel)

Category: General SOP for preparation and performing horizontal gel electrophoresis in Genetics laboratory.
Title: Performing the horizontal gel electrophoresis for extracted DNA samples and PCR products in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Post-PCR method -03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Detailed Instructions:

Important: This procedure should be carried out wearing sterile gloves

1. For 1% gel preparation, dissolve 1g of agarose in 100mL of 1X TAE buffer in a bottle by heating the solution in microwave oven.
2. Heat till agarose powder is fully dissolved. (i.e. till solution becomes clear).
3. Now add 5µL of Ethidium bromide in gel and mix the solution.
4. Cool agarose for about 3-4 minutes till the entire vapour is gone, keeping its cap open.
5. Pour the gel in casting tray and allow it to solidify.
6. Arrange the horizontal gel electrophoresis tray by placing the comb at least 1cm above the surface of the tray. Now place the electrode plates according to the charge i.e. red plate (positive) opposite to the well and black plate (negative) near the wells.
7. Connect the electrodes to the power pack (voltage suppliers) with the help of connecting wires to the respective plates and close the tank with the lid
8. After the gel has solidified, pour 1X TAE buffer on it (till the gel is completely covered by buffer) and wait for 10-15 mins.
9. Then remove the comb carefully such that wells should remain intact.
10. Place the solidified gel in the buffer tank.
11. Pour 1XTAE buffer in the tank such that all wells are immersed in it.
12. Load 5 µL of extracted DNA / PCR product in the well.
13. Now turn ON the voltage supplier.

14. Press Manual option key of power pack.
15. Now put 100V using the number keys.
16. Now press the RUN button.
17. Let the sample run till the bands reach at least 1/4th of the gel.
18. Press STOP button to stop running gel.
19. Now remove the gel from the tray.
20. Place the gel in and observe the bands. (Follow the SOP of Gel Documentation System)

Category: General SOP for preparation and performing horizontal gel electrophoresis in Genetics laboratory.
Title: Performing the horizontal gel electrophoresis for extracted DNA samples and PCR products in the Department of Clinical Pharmacology, K.E.M Hospital, Mumbai.
SOP No.: Post-PCR method -03
Date first effective: 1st January 2025 **Review date:** 31st December 2025
Department of Clinical Pharmacology, 1st floor, New MS building Seth GS Medical College & KEM Hospital, Parel, Mumbai 400012.

Gel Picture of amplified DNA