

Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00

Biopharmaceutical Development Program

Table of Contents

1.0	Purpose	1
2.0	Scope	1
3.0	Authority and Responsibility	1
4.0	Reagents and Materials	2
5.0	Equipment	2
6.0	Procedure	2
7.0	References and Related Documents	8
8.0	Change Summary	8

1.0 Purpose

This SOP details the describes the sample preparation and methods for operation of the **E-Gel Power Snap Electrophoresis and Snap Camera System** to produce fast and an efficient DNA agarose gel electrophoresis using bufferless E-Gel pre-cast agarose gels.

2.0 Scope

This SOP describes the materials and methods for operation of the **E-Gel Power Snap Electrophoresis and Snap Camera System** for the electrophoresis separation of DNA samples sizing range 10 bp to 5Kbp. The bufferless E-Gel pre-cast agarose gels with SYBR Safe, eliminates the use of the strong mutagen ethidium bromide and reduces UV exposure and considered safer and environmentally friendly. This SOP refers to, also include, specific information required for the capture of gel images using Snap Camera System.

3.0 Authority and Responsibility

- 3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.
- 3.2 PA is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 PA/QC personnel are responsible for the performance of this procedure.
- 3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.
- 3.5 BQA is responsible for quality oversight of this procedures.



Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00

Biopharmaceutical Development Program

4.0 Reagents and Materials

- 4.1 E-Gel with SYBR Safe DNA stain, 1% agarose, BDP PN 31413 or BDP approved equivalent
- 4.2 E-Gel with SYBR Safe DNA stain, 2% agarose, BDP PN 31414 or BDP approved equivalent
- 4.3 E-Gel with SYBR Safe DNA stain, 4% agarose, BDP PN 31415 or BDP approved equivalent
- 4.4 E-Gel 1 kb plus DNA ladder & 10X E-Gel sample loading buffer , BDP PN 31416 or BDP approved equivalent
- 4.5 E-Gel 50 bp DNA ladder & 10X E-Gel sample loading buffer, BDP PN 31417 or BDP approved equivalent
- 4.6 E-Gel Ultra Low Range DNA Ladder & 10X E-Gel sample loading buffer, BDP PN 31418 or BDP approved equivalent
- 4.7 DEPC water, BDP 30266 or BDP approved equivalent
- 4.8 Ultrapure Water or BDP approved equivalent

5.0 Equipment

- 5.1 E-Gel Power snap Electrophoresis Device and power Snap Camera
- 5.2 Water bath

6.0 Procedure

6.1 Determine the expected size range of the test samples (from 10 bp up to ~5Kb). Refer to table below for selecting the appropriate E-Gel SYBR safe agarose gel and DNA ladder.

Gel Type	% Agaros	Detection	DNA Ladder
	1%	100bp-5 kb	E-Gel 1 Kb plus DNA ladder
E- Gel with SYBR Safe DNA stain	2%	50bp-2Kb	E-Gel 50 bp DNA ladder
	4%	10bp-500bp	E-Gel Ultra low range DNA ladder

- 6.2 Preparing the Samples
 - 6.2.1 Dilute DNA samples with DEPC treated water 2-10 folds if the samples contain high salt concentration buffers (≥50mM Nacl, 100 mM KCL, 10 mM acetate ions or 10 mM EDTA.



Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00

Biopharmaceutical Development Program

6.2.2 Prepare DNA sample and the ladder with deionized water or 10 X sample loading buffer. Use the indicated amount of DNA per well for a single or multiple bands. Overloading DNA may cause poor resolution.

Gel type	% agarose	Sample with single band	Sample with Multiple band	DNA ladder	Total loading volume
E gel with SYBR Safe	1%, 2% and 4%	5-300 ng	500 ng	500 ng	20µL

For example: If two sample volumes to be run are 5 μ L and 8 μ L, make the final load volume 20 μ L for both samples. The volume difference will be made up with the 10 X E-Gel sample loading buffer and water. 2 μ L of the 10 X sample loading buffer will be required for each sample to obtain a 1X final loading buffer concentration. The remaining 13 μ L and 10 μ L, respectively, should be made up with water.

- 6.2.3 Record the final sample volume and indicate 10 X E-Gel sample loading buffer used on the Gel Lane Designation Form 22148-02 (**SOP 22148** *Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acid*).
- 6.2.4 Mix the completed samples by vortexing 3 times and spinning down in a micro-centrifuge for 10-30 seconds.
- 6.3 Sample Pre-heating
 - 6.3.1 Transfer the sample tubes to a $70^{\circ}C \pm 2^{\circ}C$ water bath for 10 minutes (\pm 1 minute) immediately prior to loading in order to reduce weak intermolecular and intramolecular interactions.

6.4 Prepare gel

- 6.4.1 Remove E-gel agarose gel from package and gently remove comb the cassette.
- 6.4.2 Insert gel cassette into the E-Gel power Snap Electrophoresis Device starting from the right edge then press down on the left side of the cassette.
- 6.4.3 Load the gel into the cassette compartment within 15 minutes after opening the package.



Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00



- 6.5 Load samples
 - 6.5.1 Load 20 µL of prepared samples. Keep all sample volumes uniform.
 - 6.5.2 Load 20 µL of prepared DNA ladder.
 - 6.5.3 Load 20 µL of 1X sample loading buffer in all empty wells.



- 6.6 Run the gel
 - 6.6.1 Press **Set up run** to start E-Gel protocol selection.
 - 6.6.2 Select the E-gel protocol corresponding to your gel type. Use the up / down arrows to navigate through the menu.



Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00

- 6.6.3 Press *Start run* to begin running the gel within 1 minute after loading.
- 6.6.4 The run stops automatically after the programmed time has elapsed.
- 6.6.5 Press *Done* to end the protocol.



- 6.7 Set Up the Camera
 - 6.7.1 The E-Gel Power Snap Camera shall be attached to the E-Gel Power snap Electrophoresis Device after the run is completed.
 - 6.7.2 Unfasten the docking connector cover.
 - 6.7.3 Align the docking connector with the camera connector.
 - 6.7.4 Lower the E-Gel power Snap Camera on top of the electrophpresis device and gently snap the camera in place.
 - 6.7.5 Once connected, the E-Gel Power Snap Camera displays a brief welcome splash screen, which changes to the home screen when it is ready to use.



Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993

Rev. 00



- 6.8 View and Capture image and adjust capture setting
 - 6.8.1 Check the gel by activating the Back light button. Press *View Gel* to access the view gel screen and visualize the bands on the gel.
 - 6.8.2 Adjust exposure setting if necessary.
 - 6.8.3 Four setting "Brightness, Contrast, Invert and Grayscale" of the E-Gel power Snap Camera can be adjusted during the capture session. Press Edit from the capture screen. Select the desired image setting from the drop down menu. Use + / or move the slider to adjust the selected setting. Press Done to confirm the change. Press capture to capture the image with the new setting.
 - 6.8.4 Press *Capture* to access the capture screen and save image(s) to the camera.

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Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00

- 6.9 Export image from capture screen
 - 6.9.1 Insert a USB storage device into the USB port at the front of the E-Gel Power Snap Camera.
 - 6.9.2 Press *Export* from the capture screen.
 - 6.9.3 Review the images in the active session gallery and select files for export.
 - 6.9.4 Select *Edit info* to change the file name, file type (Jpeg, TIFF or VIT format) or add comments.
 - 6.9.5 Press *Export* to export active session images to the USB storage device.



- 6.10 Export image from image gallery
 - 6.10.1 Insert a USB storage device into the USB port at the front of the E-Gel Power Snap Camera.
 - 6.10.2 Press *Gallery* from the home screen.
 - 6.10.3 Select *Thumbnails* or *list view* for navigation.
 - 6.10.4 Select **Sort** to organize files by date or file type.
 - 6.10.5 Press an image(s) to select the file or press again to de-select the file.
 - 6.10.6 Select *Actions* from the gallery screen.
 - 6.10.7 Select Edit info to change the file name, file type (Jpeg, TIFF or VIT format) or add comments.
 - 6.10.8 Press *Export* to export active session images to the USB storage device. The data should be saved to the appropriate network folder. To save the acquired data, click the Save File button. Once the Save File dialog box appears, select (S:) from the drop-down box in order to save the data on the network. Click on the PA Public Folder. Data is saved in the "E-gel capture image" Folder using an appropriate filename. Typically, the data is saved using the QC Test Request number. Click on the OK button to save the data.



Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System

SOP 22993 Rev. 00

Biopharmaceutical Development Program

6.11 Record using of the E-Gel Power Snap Electrophoresis and Snap Camera System in the log book.

7.0 References and Related Documents

E-Gel Power Snap Electrophoresis System User Guide

SOP 21531 Equipment Logs

Form 22148-02 Gel Lane Designation

SOP 22148 Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acid

8.0 Change Summary

