Standard Operating Procedure

Title: Restriction Endonuclease Enzyme Digestion of Plasmid DNA

SOP Number: 22149 **Revision Number: 01**

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Originator/Date: [redacted]

Approval/Date: [redacted]

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1.0 Purpose

This procedure describes how to digest purified plasmid DNA with various restriction endonucleases. Plasmid DNA is cut into various length DNA pieces by restriction endonuclease enzymes using various buffer and salt conditions. These restriction fragments can then be analyzed on agarose gels per **SOP 22148, *Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acids*** and **SOP 22120, *Digital Gel Imaging Using the Kodak 400 Image System***. Restriction endonucleases recognize short DNA sequences, and then cleave double-stranded DNA at specific sites within or adjacent to the recognition sequences. Restriction endonuclease cleavage of DNA into discrete fragments is one of the most basic procedures in molecular biology. The basic protocol describes how to cleave DNA for any enzyme and buffer condition. These include digesting a given DNA sample with more than one endonuclease, and digesting multiple DNA samples with the same endonuclease.

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2.0 Scope

The generic SOP refers to DNA samples requiring restriction endonuclease digestion by members of the NCI-BDP, including the Process Analytics group. Specific reactions conditions and enzymes required by a product assay profile should be indicated in a product/process specific SOP, by the product development group, or in the product assay profile itself. The procedures provided in this SOP can be used directly or as an example of how to perform any restriction endonuclease digestion of double stranded (ds DNA). Use the attached forms provided in this SOP whenever restriction digestion is performed by BDP PA.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics (PA) has the authority to define this procedure.

3.2 The Director, PA is responsible for assignment of this procedure and training of personnel.

3.3 Technicians performing this assay are responsible for following this procedure and for documenting appropriately to the Supervisor of PA, or designee.

3.4 The Supervisor, PA, or designee, is responsible for submitting reviewed and approved test data to Biopharmaceutical Quality Assurance (BQA) for CGMP projects related to testing (or to the appropriate requestor).

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Digestion of DNA with Restriction Endonucleases

Restriction endonuclease cleavage is accomplished simply by incubating the appropriate enzyme(s) with the DNA in appropriate reaction conditions. The amounts of enzyme and DNA, the buffer and ionic concentrations, and the temperature and duration of the reaction will vary depending upon the specific application.

4.1 Materials

Appropriate DNA control
10X restriction endonuclease buffers (usually supplied by the vendor of the restriction endonucleases)
Restriction endonucleases
Gel loading solution 10X (Gel stop solution) BDP PN 30177, or BDP approved equivalent.
Water bath (adjustable temperatures) or heating block (adjustable temperatures)
Sterile microcentrifuge tubes BDP PN 21640, or BDP approved equivalent.
Sterile aerosol barrier pipet tips BDP PN 26006, or BDP approved equivalent.

4.2 (***Optional***) Determination of Plasmid DNA Concentration if unknown (**SOP 22158, *Operation of the Beckman DU Series 600 Spectrophotometer***).

4.2.1 Determine A_{260} and A_{280} of a 0.1, 0.5 or 1 mL final volume using a 1:20 or 1:40 dilution by using a spectrophotometer. Record all information on Form 22158-01.

Note: Additional dilutions may be necessary to obtain an A_{260} between 0.1 and 1.0 O.D. $_{260}$. O.D. $_{260}$ values outside this range are not valid.

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4.2.2 Set A_{260} O.D. to zero with dH_2O as the blanking solution according to the relevant instrument SOP, typically **SOP 22158, *Operation of the Beckman DU Series 600 Spectrophotometer*.**

4.2.3 Calculate the concentration and yield of the test sample using the same sample volume as used in the blank (typically 1 mL).

4.2.4 O.D. A_{260} x 50 x Dilution Factor (e.g., 100) = μg/mL of undiluted sample. Record on Attachment 1.

4.3 Preparation of Restriction Endonuclease Reaction

Keeping enzymes on ice and using a new tip for each aliquoted reagent, pipet the following into a clean microcentrifuge tube. Record reagent information on Form 22158-01.

Example:

X μL DNA (0.1 to 4 μg DNA in H_2O or TE buffer);
2 μL 10X restriction buffer;
16 – X μL DNA (from above) = μL of dH_2O to add to reaction

4.4 Add restriction endonuclease (typically, 1 to 10 U/μg DNA), record and incubate the reaction mixture at least one hour at the recommended temperature (usually 37°C ± 2).

4.5 Stop the reaction by adding 1/10 volume of gel loading solution and mixing. Load gel immediately, or place at –10 to -30°C for up to two weeks.

5.0 Digesting DNA with Multiple Restriction Endonucleases

5.1 It is often desired to cleave a given DNA sample with more than one endonuclease. Two or more enzymes may be added to the same reaction mixture if all are relatively active in the same buffer and at the same temperature. Many enzymes are active in a wide variety of buffer solutions. It is frequently possible to choose a buffer solution in which two or more enzymes will retain activity. However, if the reaction conditions are too dissimilar, follow the manufacturer's recommendations.

5.2 Double digest manufactures buffers

5.2.1 Many Restriction enzyme manufacturers can supply buffers specifically designed for double or multiple digests (ex. Fermentas y - tango buffer). Follow the manufacturer's recommendations when using these buffers.

5.3 Record enzyme information and note double digests in comment section of form 22149-01.

6.0 Digesting Multiple Samples of NDA

6.1 This procedure minimizes the number of pipetting steps when multiple samples are to be digested with the same enzyme(s) and, hence, saves time. More importantly, by minimizing the number of uses of the tube containing the restriction enzyme, the potential for contamination of the enzyme stock is reduced.

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6.2 For each sample to be tested, add a constant mass and/or volume of DNA to a separate microcentrifuge tube.

6.3 Prepare a “premix solution” containing sufficient 10X restriction endonuclease buffer and water for digesting all the samples. Place the solution on ice.

6.4 Add the amount of restriction endonuclease(s) for digesting all the samples according to the assay profile or product-specific SOP.

Note: The solution to which the enzyme is added should not be more concentrated than 3X enzyme buffer to prevent loss of activity.

6.5 Add the amount of solution containing the restriction endonuclease according to the assay profile or product-specific SOP to each tube of DNA and incubate the reactions at the appropriate temperature.

6.6 Record use of a “premix solution” in Form 22149-01 comments section.

7.0 Recordkeeping

7.1 Record Spectrophotometric readings on Form 22158-01 as necessary.

7.2 Record reagent DNA on Form 22149-01

7.3 Attach Form 22149-01 to the relevant notebook, report, or PA request.

8.0 References and Related Documents

8.1 **SOP 22120** *Digital Gel Imaging Using the Kodak 400 Image Station*

8.2 **SOP 22148** *Agarose Gel Electrophoresis (A.G.E.) and Detection of Nucleic Acids*

8.3 **SOP 22158** *Operation of the Beckman DU Series 600 Spectrophotometer*

9.0 Attachments

9.1 **Attachment 1** Form 22149-01, Reagent List

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Attachment 1

NCI-Frederick
Form No.: 22149-01
SOP No.: 22149
Revision 01:

REAGENT LIST

QC Request Number(s) _____

REAGENT	BRAND NAME	PART NUMBER	LOT NUMBER	EXPIRATION DATE
Enzyme				
Buffer				
Enzyme				
Buffer				
Enzyme				
Buffer				
Enzyme				
Buffer				
Enzyme				
Buffer				
Water				

Restriction Digest set up in µl:

ENZYME	SAMPLE DNA	WATER	BUFFER	ENZYME	TOTAL	TIME	TEMP

Pipettor ID: _____

Incubator ID: _____

Comments: _____

_____

Performed By/Date: _____

Reviewed By/Date: _____

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