Benzonase® Activity Verification Assay

SOP 23120 Rev. 02

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1.0 Purpose

This procedure describes the analytical methods required to verify the presence of nuclease (DNase) activity of Benzonase® for raw material release.

2.0 Scope

The methods in this SOP are performed by Process Analytics (PA) personnel in support of raw material release testing of Benzonase® nuclease. The method described by this SOP provides a qualitative result for the presence of Benzonase® nuclease activity via digestion of a plasmid DNA substrate. This method is not intended to generate a quantitative unit concentration value.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics (PA) 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 personnel are responsible for performance of this procedure.

3.4 PA is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Reagents

Reagent	Manufacturer	BDP PN
1. 10 X Y + Tango	Fermentas	30509 (Buffer BY5)
2. Plasmid DNA pNGVL4a-CRT/E7(detox)	BDP	50164 (or equivalent)
3. DEPC Treated Water	Quality Biological	30266

5.0 Equipment

5.1 Water bath

6.0 Procedure

6.1 Dilute Benzonase® (typically provided ≥ 250 units/uL) in DEPC Water to maximum concentrations of 1 and 0.1 unit/µL.

6.2 Digest 1.9 µg of supercoiled plasmid DNA with 0, 1, and 0.1 units of Benzonase® in a final volume of 50-100 uL per reaction in a 1X Y + Tango buffer reaction. Suitable plasmid DNAs include pNGVL4a-CRT/E7 (detox, and other equivalent supercoiled constructs. The plasmid DNA used must have a concentration ≥ 1 µg/uL in order to reduce reaction volumes. Record the reagent details and digestion parameters using form 23120-01.

6.3 Incubate the reaction in a 37 ± 2°C water bath for 30 minutes ± 2 minutes. Record the incubation times on Form 23120-01.

6.4 Stop the reaction by adding 20 uL of gel loading buffer.

6.5 **Follow SOP 22993, *Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System*** for operation of the electrophoresis and capture an image of the gel.

7.0 Analysis

7.1 Control markers and ladders must exhibit clear band patterns with minimal smearing or gel electrophoresis artifacts.

7.2 The negative control lane(s) must exhibit clear scDNA and ocDNA bands with little or no smearing from low molecular weight products. Observation of distinct bands not corresponding to open circle or supercoiled DNA forms are acceptable (e.g., linear and HMW DNA forms).

7.3 Decreasing molecular weight smearing of the plasmid DNA must be observed in at least the 1 unit/mL reaction for the Benzonase® test article to be considered active. Nuclease digestion does not have to be complete for either the 1 or 0.1 unit/mL reaction to be considered positive for Benzonase® activity – i.e., the plasmid scDNA and ocDNA forms may still be present on the gel despite visible smearing.

8.0 Documentation

8.1 Record equipment, reagents, materials, part numbers, lot numbers, expiration dates, and procedures on **Form 23120-01.**

8.2 Record gel lane designations on Form 22148-02.

9.0 References and Related Documents

SOP 22993 ***Agarose Gel Electrophoresis and Detection of Nucleic acids using E-GEL Power Snap Electrophoresis and Snap Camera System***

Form 23120