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# ANALYTICAL METHOD VALIDATION REPORT: DNASE (ENDONUCLEASE) ASSAY

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## 1. PURPOSE:

1.1. To ensure the method of analysis for evaluating enzymatic activity of DNase (for nicking capability) by electrophoresis is adequately evaluated and reported for suitability of use to detect levels of 1 Unit/g in 10N Sodium Hydroxide.

## 2. SCOPE:

2.1. Applies to all 10N Sodium Hydroxide containing a specification for DNase (Endonuclease). The protocol referenced by this report may be executed multiple times to accommodate new or changing specifications. The limit of analysis in this report is 1 Unit/g.

2.2. The sample is incubated for a period of time with the substrate (plasmid pBR322). The integrity of the substrate and the presence of any degradation products are examined using agarose gel electrophoresis containing ethidium bromide stain. DNase (Endonuclease or 'nicking activity') will cause breakdown of the relatively fast migrating, supercoiled, circular pBR322 plasmid DNA to the relatively slow migrating nicked ("relaxed", but still circular) form and finally to the intermediate, migrating linear form.

2.3. For a Category 2 limit test, it is only necessary to show that a compound of interest is either present or not above and below the limit of 1 Unit/g. Specificity and Detection Limits will be assessed to validate the procedure for routine use.

## 3. RESPONSIBILITIES:

3.1. The Senior Product Life Cycle Manager is responsible for the implementation, control and maintenance of this report.

## 4. REFERENCES:

4.1. BMV01 pp43-46

4.2. BSI-PRL-0858, Analytical Method Validation Protocol: DNase (Endonuclease) Assay for 10N Sodium Hydroxide

4.3. BSI-SOP-0098, Balance SOP

4.4. BSI-SOP-0134. Pipette SOP

4.5. BSI-SOP-0135, Laboratory Chemicals

4.6. BSI-SOP-0259, Fisher Scientific Isotemp Water Bath Operation Calibration SOP

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## 5. EQUIPMENT:

**TABLE 1: EQUIPMENT**

| Equipment | Serial Number | Calibration Due | Manufacturer | Date of Last Calibration |
|---|---|---|---|---|
| Analytical Balance | 24801744 | 4/30/25 | Sartorius | 10/4/24 |
| 500µL - 5000µL Pipette | H33986M | 2/28/25 | Eppendorf Research Plus | 8/6/24 |
| 100µL - 1000µL Pipette | R14419C | 2/28/25 | Eppendorf Research Plus | 8/6/24 |
| 20µL - 200µL Pipette | N41555G | 3/31/25 | Eppendorf Research Plus | 9/9/24 |
| 2µL - 20µL Pipette | R12216C | 6/30/25 | Eppendorf Research Plus | 12/10/24 |
| Calibrated Timer | 230665671 | 9/29/25 | FisherBrand | 9/29/23 |
| Centrifuge | 41650138 | Not Applicable | Fisher Scientific accuSpin Micro 17 | Not Applicable |
| Water Bath | 300004011 | 7/25 | Fisher Scientific Isotemp | 7/22/24 |
| E-Gel Power Snap Electrophoresis Device | 2848022120117 | Not Applicable | Invitrogen | Not Applicable |
| E-Gel Power Snap Camera | 2848122060090 | Not Applicable | Invitrogen | Not Applicable |
| 2.5mL Centrifuge Tubes | Part No. 780540 | Not Applicable | Fisher Scientific | Not Applicable |

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## 6. REAGENTS:

| TABLE 2: REAGENTS | | | | | |
|---|---|---|---|---|---|
| **Reagent** | **Lot Number** | **Expiration Date** | **Manufacturer** | **Date of Opening** | **Part Number** |
| DNase I Enzyme (251.5Unit/μL) | 2813075 | 5/28/26 | Invitrogen | 3/25/24 | 18047019 |
| DNase I Buffer | BSP44P89 | 4/18/25 | In-House Solution | 12/18/24 | Not Applicable |
| HEPES | SLCM4091 | 1/31/26 | Sigma Aldrich | 7/8/24 | PHG0001-100G |
| pBR 322 DNA Substrate | 2441895 | 4/24/25 | Invitrogen | 4/24/24 | 15367-014 |
| TE Buffer | BSP44P49 | 2/28/25 | In-House Solution | 10/29/24 | Not Applicable |
| DNase 10x Reaction Buffer | BSP44P48 | 1/22/25 | In-House Solution | 10/22/24 | Not Applicable |
| Sterile Water | 6402006 | 2/26 | Ricca | 1/21/25 | R9145000-1G |
| Gel Loading Buffer | BSP44P68 | 1/27/25 | In-House Solution | 11/27/24 | Not Applicable |
| 1% Agarose E-Gel Cassette | T300724-01 | 7/30/25 | Invitrogen | 1/22/25 | G401001 |
| 10N NaOH | NAHY-M03-0125-0003 | Not Applicable | BioSpectra Inc. | 1/12/25 | Not Applicable |

## 7. VALIDATION PROCEDURE:

7.1. Assay

7.1.1. Prepared each sample (typically 2% or 0.02 g/mL) utilizing the table below, in triplicate:

| TABLE 3: VALIDATION SAMPLE / SPIKE SOLUTION PREPARATION | | | | |
|---|---|---|---|---|
| **Sample ID** | **Sample Volume (μL)[1]** | **HEPES Weight (g)** | **DNase 1 Buffer Volume (μL)** | **Volume of Stock Solution B (μL)** |
| 0% Spike 1 | 15 | 0.0771 | 985 | 0 |
| 0% Spike 2 | 15 | 0.0716 | 985 | 0 |
| 0% Spike 3 | 15 | 0.0729 | 985 | 0 |
| 100% Limit Spike 1 | 15 | 0.0722 | 975 | 10 |
| 100% Limit Spike 2 | 15 | 0.0708 | 975 | 10 |
| 100% Limit Spike 3 | 15 | 0.0730 | 975 | 10 |

[1]Equivalent to 0.02g based on density of 1.33g/mL for 10N NaOH

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7.1.2. Prepared standards utilizing the table below:

| **TABLE 4: DNASE (ENDONUCLEASE) STANDARDS PREPARATION** | | | |
|---|---|---|---|
| **Purpose** | **Final Concentration (Unit/µL)** | **Volume of DNase I Enzyme (µL)** | **Volume of DNase I Buffer (µL)** |
| **Stock Solution A** | **0.2** | 1.69 of DNase I | 2118.31[1] |
| **Stock Solution B** | **$0.2x10^{-2}$** | 10 of 0.2 | 990 |
| **100% Limit Std.** | **$0.2x10^{-4}$** | 10 of $0.2x10^{-2}$ | 990 |

[1]Volume of DNase I buffer for DNase I enzyme at 251.5 Units/µL

7.1.3. Diluted Substrate prior to preparing reaction mix, as follows:

| **TABLE 5: DNASE (ENDONUCLEASE) SUBSTRATE PREPARATION** | | |
|---|---|---|
| **Final Concentration (µg/µL)** | **Volume of pBR 322 DNA Substrate (µL)** | **Volume of TE Buffer (µL)** |
| 0.1 | 8 | 12 |

7.1.4. Prepared a Reaction Mix, where Y represents the total number of tubes to be prepared, as follows:

| **TABLE 6: ENDONUCLEASE REACTION MIX** | |
|---|---|
| **Amount** | **Solution** |
| (Y+1) x 1µL: 20µL | Diluted pBR322 DNA Substrate |
| (Y+1) x 1µL: 20µL | DNase 10x Reaction Buffer (Endonuclease) |
| (Y+1) x 3µL: 60µL | Sterile Water |

7.1.5. Labelled an appropriate number of microcentrifuge tubes and add previously prepared solutions to each of the tubes as follows:

| **TABLE 7: REACTION SCHEME** | | | | | |
|---|---|---|---|---|---|
| | **Blank** | **Test Solution** | **100% Spike Sample** | **100% Limit Standard** | **Control** |
| **Tube #** | **1** | **2** | **3** | **4** | **5** |
| Reaction Mix (µL) | 5 | 5 | 5 | 5 | 5 |
| DNase 1 Buffer (µL) | 5 | - | - | - | 5 |
| Test Solution (µL) | - | 5 | 5 | - | - |
| Control Enzyme[1] (µL) | - | - | - | 5[1] | - |

[1]Appropriately diluted DNase I. (Note, for instance, that 5 microliters of $0.2 \times 10^{-4}$ Units DNase/µL represents $1 \times 10^{-4}$ Units DNase.)

7.1.6. Mixed thoroughly and immediately placed the Control onto ice or into a temperature monitored refrigerator.
7.1.7. Incubated all others at 37°C for 4 hours.
7.1.8. Cooled tubes on ice or in a temperature monitored refrigerator for approximately 5 minutes.
7.1.9. Centrifuged all tubes for 1 minute.
7.1.10. To each tube, added 4µL of Gel Loading Buffer.
7.1.11. Vortexed thoroughly.
7.1.12. Centrifuged for 1 minute.

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7.2. Electrophoresis

7.2.1. Utilized 1% Agarose E-GEL cassettes.

7.2.2. Removed the E-GEL cassette it's from packaging, carefully remove the well comb, and inspect under sufficient lighting.

7.2.2.1. Inspected for any damage to the wells and any defects. If any damage is found, discard properly.

7.2.2.2. Note: Loaded the gel within 15 minutes of opening the package and ran gel within one minute of loading the samples

7.2.3. Placed the E-GEL cassette into the E-Gel Power Snap Electrophoresis Device starting from the right edge.

7.2.4. Loaded the entire sample into to the well.

7.2.5. Loaded all empty wells with 14µL with Sterile Water.

7.2.6. Set up the run by selecting the E-Gel Protocol 1-2% on the E-Gel Power Snap Electrophoresis Device.

7.2.7. Ensured the run time was 10 min.

7.2.8. Ran the gel by pressing "Start Run"

7.3. Photograph

7.3.1. Labelled the Cassette appropriately.

7.3.1.1. Endo, Initials / Date

7.3.2. Connected the E-Gel Power Snap Camera to the Electrophoreses unit.

7.3.3. Pressed Capture in the home screen view.

7.3.3.1. Note: Allowed the gel to cool for 5-10 minutes before image capture to enhance sensitivity.

7.3.4. Exported image to a USB thumb drive.

7.3.5. Printed image with the wells labelled

## 8. VALIDATION PARAMETERS:

8.1. **System Suitability:**

8.1.1. All of the following system suitability acceptance criteria must be met for the assay results to be suitable for use:

8.1.1.1. The Blank lane demonstrates substrate degradation equal to or more pronounced than the Control lane.

8.1.1.2. The 100% Limit Standard lane shows substrate degradation more pronounced than the Blank lane.

8.1.1.3. The 100% Limit Standard lane substrate is visible and degraded.

8.2. **Specificity:**

8.2.1. Specificity will be demonstrated by comparing the control lane, 0% spiked sample solution lane, and 100% level spiked solution lane.

8.2.2. Acceptance Criteria:

8.2.2.1. The 100% level spike should demonstrate more pronounced degradation than the 0% spiked sample solution.

8.2.2.2. The 0% spike sample solution should demonstrate degradation equal to or more pronounced than the Control.

8.3. **Detection Limit:**

8.3.1. Report the level of detectability in DNase units per gram.

$$\text{Units/g} = (1\text{x}10^{-4}\text{Unit})/(0.005\text{mL}*0.02\text{g/mL})$$

8.3.2. Acceptance Criteria:

8.3.2.1. No less than 1 Unit/g is acceptable.

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## 9. VALIDATION RESULTS:

| TABLE 8: 10N SODIUM HYDROXIDE ENDONUCLEASE VALIDATION RESULTS | |
|---|---|
| **System Suitability** | |
| **Parameter** | **Result** |
| 0% (Blank) lane demonstrates substrate degradation equal to or more pronounced than the control lane. | Pass |
| The 100% level standard lane shows substrate degradation more pronounced than the 0% (Blank). | Pass |
| The 100% standard lane substrate is visible and degraded. | Pass |
| **Specificity** | |
| **Parameter** | **Result** |
| The 100% level spike should demonstrate more pronounced degradation than the 0% spiked sample solution. | Pass |
| The 0% spike sample solution should demonstrate degradation equal to or more pronounced than the control. | Pass |
| **Limit of Detection** | |
| **Parameter** | **Result** |
| Report the level of detectability in DNase units per gram as less than the standard. NMT 1 Units/g is acceptable. $\frac{Units}{g} = \frac{1 \times 10^{-4}\, Unit}{(0.005mL \times 0.02\frac{g}{mL})}$ | 1 Units/gram |

## 10. CONCLUSION:

10.1. The method “DNase (Endonuclease) Assay” is considered validated and suitable for use for 10N NaOH at all BioSpectra, PA facilities. All acceptance criteria for System Suitability, Specificity, and Limit of Detection were met. The Limit of Detection was determined to be 1 Units/gram.

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