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ANALYTICAL METHOD VALIDATION REPORT: RNASE (RIBONUCLEASE) ASSAY FOR 10N SODIUM HYDROXIDE

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1. PURPOSE:

1.1. To ensure the method of analysis for evaluating enzymatic activity of RNase 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 RNase. This 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. 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 control, implementation, and maintenance of this report.

4. REFERENCE:

4.1. BMV01 pp48-51
4.2. BSI-PRL-0859, Analytical Method Validation Protocol: RNase (Ribonuclease) 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
4.7. BSI-SOP-0436, Analytical Methods Validation Master Plan

5. MATERIALS AND 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
0.5µL - 10µL Pipette	R32893H	4/30/25	Eppendorf Research Plus	10/15/24
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
Microcentrifuge Tubes	Not Applicable	Not Applicable	FisherBrand	Not Applicable

6. REAGENTS:

TABLE 2: REAGENTS

Reagent	Lot Number	Expiration Date	Manufacturer	Date of Opening	Part Number
RNase Standard	BSP44P50	5/19/25	In-House Solution	10/29/24	Not Applicable
HEPES	SLCM4091	1/31/26	Sigma Aldrich	7/8/24	PHG0001-100G
RNase Buffer	BSP44P99	9/15/25	In-House Solution	1/15/25	Not Applicable
Millennium RNA Marker	3002074	3/31/27	Invitrogen	8/30/24	AM7150
RNase 10x Reaction Buffer	BSP45P01	4/15/25	In-House	1/15/25	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
2% Agarose E-Gel Cassette	U050524-01	5/5/25	Invitrogen	1/23/25	G401002
10N NaOH	NAHY-M03-0125-0003	Not Applicable	BioSpectra Inc.	1/12/25	Not Applicable

7. VALIDATION PROCEDURE:

7.1. Assay

7.1.1. Prepare 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)	RNase Buffer Volume (µL)	Volume of Stock Solution B (µL)
0% Spike 1	15	0.0722	985	0
0% Spike 2	15	0.0709	985	0
0% Spike 3	15	0.0747	985	0
100% Limit Spike 1	15	0.0741	885	100
100% Limit Spike 2	15	0.0700	885	100
100% Limit Spike 3	15	0.0723	885	100

[1]Equivalent to 0.02g based on 10N NaOH density of 1.3g/mL.

7.1.2. Prepare standards utilizing the table below:

TABLE 4: RNASE (RIBONUCLEASE) STANDARDS PREPARATION			
Purpose	**Final Concentration (Unit/µL)**	**Volume of RNase Solution[1](µL)**	**Volume of RNase Buffer (µL)**
Stock Solution A	**$1x10^{-3}$**	2.30 of RNase Solution[2]	1997.70[2]
Stock Solution B	**$0.2x10^{-3}$**	200 of 0.2	800
100% Limit Std.	**$0.2x10^{-4}$**	100 of $0.2x10^{-2}$	900

[1]10 mg/mL

[2]Volumes based on the concentration reported on the RNase vendor's C of A; 87U/mg.

7.1.2.1. **Note:** RNase (Ribonuclease) Standard preparation is dependent on RNase (Ribonuclease A) enzyme activity (found on the Certificate of Analysis or on the reagent container). The volume of RNase Solution used was determined using the following equation (may be scaled as needed):

$$Volume\ of\ RNase\ Solution\ (\mu L) = \frac{\left(1 \times 10^{-3}\frac{Units}{\mu L}\right) \times \left(Final\ Volume\ (\mu L)\right)}{\left(RNase\ Solution\ Concentration\ \left(\frac{mg}{\mu L}\right)\right) \times \left(RNase\ Enzyme\ Activity\ \left(\frac{Units}{mg}\right)\right)}$$

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

TABLE 5: RIBONUCLEASE REACTION MIX	
Amount	**Solution**
(Y+1) x 1µL: 10µL	Millennium RNA Marker
(Y+1) x 1µL: 10µL	RNase 10x Reaction Buffer
(Y+1) x 3µL: 30µL	Sterile Water

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

TABLE 6: 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
RNase Buffer (µL)	5	-	-	-	5
Test Solution (µL)	-	5	5	-	-
Control Enzyme[1] (µL)	-	-	-	5[1]	-

[1]Appropriately diluted RNase. (Note, for instance, that 5 microliters of 0.2×10^{-4} Units RNase per microliter represents 1×10^{-4} Units RNase.)

7.1.5. Mix thoroughly and immediately place the Control onto ice or into a temperature monitored refrigerator.

7.1.6. Incubate all others at 37°C for 4 hours.

7.1.7. Cooled tubes on ice or in a temperature monitored refrigerator for approximately 5 minutes.

7.1.8. Centrifuged all tubes for 1 minute.

7.1.9. To each tube, added 4µL of Gel Loading Buffer.

7.1.10. Vortexed thoroughly.

7.1.11. Centrifuged for 1 minute.

7.2. Electrophoresis

7.2.1. Utilized 2% Agarose E-GEL cassettes.

7.2.2. Removed the E-GEL cassette from its packaging, carefully removed the well comb, and inspected under sufficient lighting.

7.2.2.1. Inspected for any damage to the wells and any defects.

7.2.2.2. **Note:** Loaded the gel within 15 minutes of opening the package and run gel within one min of loading the samples

7.2.3. Inserted the 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 of 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 is 10 minutes.

7.2.8. Ran the gel by pressing "Start Run".

7.3. Photograph

7.3.1. Label the cassette appropriately.

7.3.1.1. RNase, 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: Allow 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 labeled.

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.

8.2. **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 spiked sample 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 RNase 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.

9. VALIDATION RESULTS:

TABLE 7: 10N SODIUM HYDROXIDE RNASE 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
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 RNase units per gram. 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 "RNase 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.