MANUAL

DNAse Activity Assay

Catalog #: NA-DNASE-TOT

RayBiotech
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ISO 13485:2016

Introduction

The DNAse Activity Assay offers a quick, sensitive, and straightforward approach to measuring total nuclease activity using a compatible Real-Time PCR instrument. This kit tracks the increase in FAM fluorescence produced from the degradation of the FAM-labeled double-stranded DNA (dsDNA) hydrolysis probe (as shown in Figure 1) by nucleases in the sample. The assay is capable of detecting nuclease activity ≤ $1.03x10^{-4}$ Units.

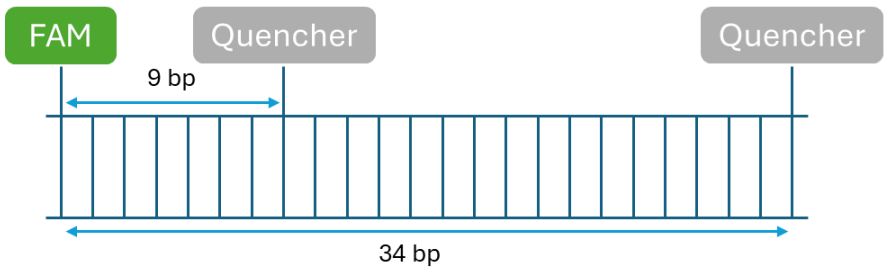

Figure 1. Double stranded DNA hydrolysis probe.

Kit Components

Store the kit components according to storage temperatures in the following table for up to 3 months. Protect dsDNA Probe (NA-TOT-DSP) from light. Kit must be used within expiry period to ensure optimal performance. Do not mix reagents from different kit lots.

CATALOG NUMBER	COMPONENT	96 TESTS	2 x 96 TESTS	Storage
NA-TOT-DSP	dsDNA Probe (50X)	2 x 60 µL vials	4 x 60 µL vials	≤-20°C
NA-TOT-STD	Lyophilized Nuclease Standard (0.5 Units)	2 vials	4 vials	
NA-TOT-CB	Cleavage Buffer (10X)	1 x 1.2 mL vial		4°C
NA-TOT-SD	Sample Diluent (2X)	1 x 15 mL bottle		
	Optical Seal	1 seal	2 seals	RT

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Required Materials (NOT INCLUDED)

1. Vortex Mixer
2. Tabletop centrifuge
3. Pipettes and nuclease-free pipette tips (barrier tips recommended)
4. Sterile RNase/DNAse free cotton swabs (if testing environmental swabs)
5. Nuclease free dH_2O
6. Plate reader or PCR thermocycler with following capabilities:
    a. Reading at 494nm excitation and 518nm emission
    b. Temperature control
    c. Optional: Real-time data collection
7. Nuclease free 96-Well ELISA or PCR plate

General Considerations

1. Read this manual carefully before the experiment.
2. Allow assay components to equilibrate to room temperature before use.
3. Use calibrated pipettes and nuclease-free centrifuge tubes, conical tubes, and pipette tips.
4. Tests must be run at least in duplicate.

Assay Procedure

1. Prepare Master Mix:
    a. Determine total # of tests to prepare:

    $$\boldsymbol{Number\ Reactions} = (\#\boldsymbol{Samples} + \#\boldsymbol{Standards}) \times \#\ \boldsymbol{Replicates} \times \mathbf{1.2}$$ *(round up)*

    b. Prepare Master mix by mixing the following components according to following table:

Component	Volume
dsDNA Probe	1 µL
10X Cleavage Buffer	5 µL
Nuclease free dH_2O	19 µL
Total	25 µL

    ***Note**: Scale up based on number of tests calculated in Step 1b above.*

    c. Pulse vortex at medium speed 3 to 5 times.

2. Prepare Standard Curve:

   ***Note**: Units of nuclease activity for each standard reaction are as follows (from Standard 1 to Standard 6): $2.5x10^{-2}$-, $8.33x10^{-3}$-, $2.78x10^{-3}$-, $9.26x10^{-4}$-, $3.09x10^{-4}$-, and $1.03x10^{-4}$-Units/reaction. An optional seventh standard can be prepared to measure nuclease activity as low as $3.43x10^{-5}$. This will require a longer incubation that may reduce quality of top standard.*

   a. Prepare 1X Sample Diluent by combining 750 µL 2X Sample Diluent with 750 µL Nuclease free dH2O.
   b. Label a series of 1.5 mL centrifuge tubes “Standard 2” to “Standard 6.” Label a 7th tube “Blank.”
   c. Add 100 µL 1X Sample Diluent into each of the above labeled vials.
   d. Reconstitute the lyophilized nuclease standard to 0.001 Units/uL with 500 uL 1X Sample Diluent. This will serve as standard 1
   e. Prepare standard 2 by transferring 50 µL from standard 1 into tube labeled “Standard 2.” Mix well by pipetting (do not vortex).
   f. Repeat for each serial dilution to prepare the remaining standards.
   g. The tube labeled “Blank” will serve as the test blank.
   h. Store prepared standards and blank on ice until ready to use (≤20 minutes).

3. Prepare Samples:

   a. Liquid Samples

      ***Note**: Recommended that a vehicle blank be run with buffer used to prepare samples.*

      i. Dilute samples 2-fold with 2X Sample Diluent.

         ***Note**: If higher sample concentrations are required, dilute samples in advance prior to diluting with 2X Sample Diluent. The total dilution will be the prior dilution multiplied by 2.*

      ii. Higher dilutions may be appropriate depending on sample type and expected nuclease activity.

   b. Swabs

      ***Note**: Recommended that a swab blank be run with a clean, unused swab.*

      i. Dilute an appropriate volume of 2X Sample Diluent 2-fold with Nuclease free dH2O to prepare 1X Sample Diluent working stock.
      ii. Transfer 100 µL of the 1X Sample Diluent to labeled sterile, RNAse/DNAse free tubes.

iii. Saturate the tip of a sterile, RNAse/DNAse free cotton swab with 1X Sample Diluent by dipping into one of the labeled tubes.

iv. Swab area to be tested corresponding to labeled tube for 5 – 10 seconds. ***Note****: Skip this step for the swab blank.*

v. Place cotton swab back into the same test tube (from step iii) and swirl for 5 to 10 seconds.

vi. Discard cotton swab.

vii. Repeat steps iii to vi for all remaining samples.

viii. Cap tubes and store on ice until ready to test (do not exceed 20 minutes).

5. Prepare Reactions:

a. Prepare plate map as appropriate for samples to be tested. An example is provided below.

	1	2	3	4	5	6
A	Standard 1			Sample 1		
B	Standard 2			Sample 2		
C	Standard 3			Sample 3		
D	Standard 4			Sample 4		
E	Standard 5			Sample 5		
F	Standard 6			Sample 6		
G	Blank			Sample 7		
H	Vehicle Blank			Sample 8		

b. If measuring with an ELISA plate reader and a 96-Well ELISA plate, add 50 µL nuclease free dH_2O to all wells. Skip if using 96-Well qPCR plates.

c. Add 25 µL Master Mix to each well of the 96-well plate

d. Add 25 µL standard or sample to indicated wells of the 96-well plate. Mix well.

e. Seal plate with optical film.

6. Measure Fluorescence:

a. If applicable, set sample volume to 50 µL or 100 µL (depending on plate).

b. Fluorescence measurement settings:

i. Sample volume: 50 µL or 100 µL (depending on plate)

ii. Excitation: 494nm

iii. Emission: 518nm

iv. Gain: Set gain to autoscale (if possible).

v. Temperature: 37ºC

c. Place plate into a PCR thermocycler or plate reader.
d. Incubate samples at 37°C and measure fluorescence every 60 seconds for 60 to 90 minutes. If instrument is not capable of real time data collection, manually measure fluorescence at the start of reaction and at 5-minute intervals.

Data Analysis

1. Normalize fluorescence data for well A1 by subtracting initial fluorescence measurement from all other measurements of that well.
2. Repeat Step 1 for all remaining wells.
3. Determine optimal time point to serve as end-point fluorescence data.
   Note 1*: This step often requires some optimization for each instrument.*
   Note 2*: Shorter incubation times often provide better resolution of higher nuclease activities, whereas longer incubation times are better able to resolve lower activities.*
4. Calculate average normalized fluorescence of all standards and samples at chosen endpoint.
5. Plot average normalized fluorescence of standards versus Units of nuclease activity.
6. Model data with appropriate regression model and use best fit curve to extrapolate nuclease activities of unknown samples.
7. Calculate nuclease activities of undilute samples by multiplying extrapolated volumes by dilution factor.

Assay Performance

Assay performance was assessed across three replicates performed on multiple days.