Technical Bulletin

# Neuraminidase Assay Kit

**Catalogue number MAK121**

## Product Description

Neuraminidase, also known as Sialidase, is an enzyme that hydrolyzes terminal sialic acid residues on poly-saccharide chains. It is predominantly expressed in microorganisms such as bacteria and viruses. Cleavage of sialic acid residues by neuraminidase is believed to play several roles in infection by influenza viruses. It is thought to assist in the penetration of mucosal linings, the invasion of target cells, the elution of progeny viruses from infected cells, and the prevention of self-aggregation. Thus, neuraminidase is an important target for influenza drug development and simple, direct, and automation-ready procedures for measuring neuraminidase activity find wide applications in research and drug discovery.

The Neuraminidase Assay Kit is suitable for the measurement of neuraminidase activity in biological samples and for the screening of neuraminidase inhibitors. In this assay, neuraminidase activity is determined by an enzymatic assay in which sialic acid released by neuraminidase results in a colorimetric (570 nm)/fluorometric ($\lambda_{ex}$ = 530/$\lambda_{em}$ = 585 nm) product directly proportional to neuraminidase activity in the sample. The linear detection range at 37 °C is 0.1–10 units/L for colorimetric assays and 0.01–2 units/L for fluorometric assays.

## Components

| | |
|---|---|
| Assay Buffer<br>Catalogue Number MAK121A | 6 mL |
| Dye Reagent<br>Catalogue Number MAK121B | 60 µL |
| Substrate<br>Catalogue Number MAK121C | 6 mL |
| Enzyme<br>Catalogue Number MAK121D | 120 µL |
| Cofactors<br>Catalogue Number MAK121E | 120 µL |
| Standard, 10 mM<br>Catalogue Number MAK121F | 500 µL |

## Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

## Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

The kit is shipped on dry ice and storage at -20 °C, protected from light, is recommended.

## Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Equilibrate all components to the desired reaction temperature (37 °C).

## Procedure

### Standards for Colorimetric Detection

Add 20 µL of the 10 mM Standard to 480 µL of water to prepare a 400 µM Standard Working Solution. Add 0, 15, 30, and 50 µL of the 400 µM Standard Working Solution into tubes. Add water to each tube to bring the volume to 50 µL, generating 0 (blank), 120, 240, and 400 µM Standards. Transfer 20 µL of Standards into separate wells of 96 well plate.

### Standards for Fluorometric Detection

Prepare Standards as described for the Colorimetric Detection. Further dilute each Standard 5-fold with water, generating 0 (blank), 24, 48, and 80 µM Standards. Transfer 20 µL of Standards into separate wells of 96 well plate.

### Sample Preparation

Aliquot 20 µL of each Sample into two separate wells of a 96 well plate. One well will be used for the Sample activity and one for the Sample blank.

**Notes**: Thiol (SH) group containing reagents (For example- Mercaptoethanol, DTT) may interfere with this assay and should be avoided in Sample preparation.

For unknown Samples, it is suggested to test several Sample dilutions to ensure the readings are within the linear range of the Standard curve.

### Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 80 µL of the Master Reaction Mix is required for each Sample, blank, and Standard reaction (well).

**Table 1.**

Reaction Mixes

| Reagent | Sample and Standards | Sample blank |
|---|---|---|
| Assay Buffer | 30 µL | 85 µL |
| Substrate | 55 µL | – |
| Cofactors | 1 µL | 1 µL |
| Enzyme | 1 µL | 1 µL |
| Dye Reagent | 0.5 µL | 0.5 µL |

2. Add 80 µL of the appropriate reaction mix to each well. Mix well using a horizontal shaker or by pipetting and incubate the reaction at 37 °C. Protect the plate from light during the incubation.
3. Measure the absorbance of the Samples and Standards at 20 minutes at 570 nm for colorimetric assay or $\lambda_{ex}$ = 530/$\lambda_{em}$ = 585 nm for fluorometric assay to determine the $M_{20\ min}$.
4. Continue to incubate the plate at 37 °C for an additional 30 minutes. Measure the absorbance of the Samples and Standards at 570 nm for colorimetric assay or $\lambda_{ex}$ = 530/$\lambda_{em}$ = 585 nm for fluorometric assay to determine the $M_{50\ min}$.

## Results

A new standard curve must be set up each time the assay is run.

Plot the absorbance or fluorescence intensity measured at 50 minutes for each standard against the standard concentrations. Determine the slope using linear regression fitting.

Subtract the $M_{20\ min}$ value from the $M_{50\ min}$ value for the sample, sample blank, and water (standard blank) reactions to determine the change in measurement value (ΔM).

## Calculation

The neuraminidase activity (units/L) of a sample is calculated as follows:

$$\text{units/L} = \frac{\Delta M_{sample} - \Delta M_{blank} - \Delta M_{water}}{\text{Slope} \times T}$$

where:

$\Delta M_{sample}$, $\Delta M_{blank}$, $\Delta M_{water}$ = The changes in absorbance or fluorescence intensity of the sample, sample blank, and water (standard blank), respectively.

Slope = The slope of the standard curve in $\mu M^{-1}$

T = The time of reaction between readings (30 minutes)

**Note**: If the Sample activity is higher than the 10 units/L for the colorimetric assay or 2 units/L for the fluorometric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

## Troubleshooting Guide

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay not working | Cold assay buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if needed to use multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Master Reaction Mix before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the reaction mix |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare a Master Reaction Mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the plate well |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |

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