Technical Bulletin

Fluorometric Maleimide Assay Kit

Catalogue number MAK167

Product Description

Maleimide is an unsaturated imide used in protein conjugation reactions.
The Fluorometric Maleimide Assay Kit provides a sensitive, one-step fluorometric assay to detect maleimide, which reacts with a proprietary dye, resulting in a fluorometric product (λ_{Ex} = 490/λ_{Em} = 525 nm) proportional to the amount of maleimide present. The assay can be performed in either a 96 or 384 multiwell plate format using a fluorescence microplate reader.

Components

The kit is sufficient for 200 fluorometric assays in 96-well plates.

Fluorescent Maleimide Detection Reagent Catalogue Number MAK167A	1 vial
Reaction Buffer Catalogue Number MAK167B	0.5 mL
Assay Buffer Catalogue Number MAK167C	25 mL
N-ethylmaleimide Standard, 10 mM Catalogue Number MAK167D	1 vial
DMSO Catalogue Number MAK167E	0.2 mL

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescence multiwell plate reader
- Black flat-bottom 96-well plate. Cell culture or tissue culture treated plates are not recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store all components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate reagents to room temperature prior to use.

Fluorescent Maleimide Detection Reagent – Reconstitute vial with 20 µL of DMSO to generate the 500× Stock Solution. Mix well by pipetting, aliquot, and store at -20 °C any remaining Stock Solution. Stable for 1 month when stored at -20 °C.

N-ethylmaleimide Standard – Aliquot the *N*-ethylmaleimide Standard solution into single use aliquots. Store at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Preparation of 20× Reaction Buffer

To 250 µL of Reaction Buffer add 10 µL of the 500× Detection Reagent Stock Solution to generate the 20× Reaction Mixture. Mix well by pipetting. Incubate at room temperature for 30 minutes, protected from light.

Note: It is important to incubate the 20× Reaction Mixture at room temperature for at least 30 minutes to maximize the signal to background ratio. The 20× Reaction Mixture will become yellow in color.

N-ethylmaleimide Standards

1. Add 10 µL of the 10 mM *N*-ethylmaleimide Standard solution to 990 µL of Assay Buffer to prepare a 100 µM *N*-ethylmaleimide Standard solution.
2. Take the 100 µM *N*-ethylmaleimide Standard solution and further dilute it to 10 µM.
3. Prepare 1:2 serial dilutions with Assay Buffer generating 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0 (blank) µM Standards. Add 50 µL of each serially diluted Standard into appropriate wells of a 96-well plate.

Note: The 10 µM Standard may be omitted if linearity is not sufficient.

Sample Preparation

Add up to 50 µL of Sample to wells. Bring Samples to a final volume of 50 µL with Assay Buffer.

Note: 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

Set up the Master Reaction Mix according to the scheme in Table 1. Add the entire contents of the 20×Reaction Buffer Mixture to 5 mL of Assay Buffer. Mix well.

Table 1.

Master Reaction Mix

Reagent	Volume
20× Reaction Buffer Mixture	260 µL
Assay Buffer	5 mL

Note: The Master Reaction Mix is enough for one plate. If necessary, the Master Reaction Mix can be scaled down. The Master Reaction Mix should be used within 1 hour.

1. Add 50 µL/well (96 well plate) or 25 µL/well (384 well plate) of Master Reaction Mix into the cell plate. Incubate the cells for 5–30 minutes at room temperature, protected from light.
2. Measure the fluorescence intensity:

   λ_{ex} = 490/λ_{em} = 525 nm. (Cutoff = 515nm)

Results

Calculations

The background blank for the assay is the value obtained for the 0 (blank).
N-ethylmaleimide Standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from
all readings.

Use the values obtained from the Standards to plot a Standard curve. The concentration of maleimide present in the Samples may be determined from the Standard curve.

Note: A new Standard curve must be set up each time the assay is run.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak167pis Rev 12/23