3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Colorimetric Maleimide Assay Kit

Catalog Number **MAK162**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Maleimide is an unsaturated imide used in protein conjugation reactions. The direct measurement of maleimide moieties following conjugation reactions by spectrophotometric analysis at 302 nm is insensitive due to the small extinction coefficient (620 $M^{-1}cm^{-1}$) and complicated by protein absorbance at this wavelength.

The Colorimetric Maleimide Quantiation Kit provides a simple and direct procedure for measuring maleimide groups. The sample is first reacted with a known amount of excess thiol and then the remaining unreacted thiol is assayed using 4,4′-DTDP (4,4′-Dithiodipyridine). The amount of maleimide is calculated as the difference between the initial amount of thiol and the amount of unreacted thiol after the complete reaction of all maleimide groups.

Components

The kit is sufficient for 100 assays in cuvettes.

MEA (2-Aminoethanethiol Hydrochloride) Catalog Number MAK162A	1 vl
4,4′-DTDP (4,4′-Dithiodipyridine) Catalog Number MAK162B	1 vl
Assay Buffer Catalog Number MAK162C	50 mL
DMSO Catalog Number MAK162D	1 mL

Reagents and Equipment Required but Not Provided.

- Cuvettes
- Spectrophotometer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped under ambient conditions 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. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Allow all reagents to come to room temperature before use.

MEA – Reconstitute with 0.2 mL of water to make a 500× MEA Stock Solution. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

4,4′-DTDP – Reconstitute with 1 mL of DMSO to make a 50× 4,4′-DTDP Stock Solution. Mix well by pipetting (do not vortex), then aliquot and store, protected from light, at –20 °C.

Procedure

Assay Reaction

1. Set up the MEA Working Solution according to the scheme in Table 1. 100 µL of the MEA Working Solution is required for each reaction.

Table 1.
MEA Working Solution

Reagent	Volume
500× MEA Stock Solution	2 µL
ultrapure water	1 mL

Note: The MEA Working Solution is enough for 10 reactions and can be scaled as necessary.

2. Set up three Total Sulfhydryl (SH) reactions by adding 0.4 mL of Assay Buffer and 0.1 mL of MEA Working Solution into each of three tubes.

3. Set up three tubes for each sample. Into each tube add 0.05 mg of conjugate test sample, sufficient Assay Buffer to bring the volume to 0.4 mL/tube, and 0.1 mL of MEA Working Solution.

4. Incubate the tubes at room temperature for 20 minutes.

5. To determine Total SH, add 10 µL of the 50× 4,4′-DTDP Stock Solution to each of the Total SH reactions (do not add to the sample containing tubes). Incubate the tubes for 2 minutes at room temperature.

6. Measure the absorbance of Assay Buffer only at 324 nm (A_{324}) as a blank control. Measure the A_{324} of the three Total SH reactions. It is not necessary to wash the cuvette between readings of the Total SH reaction replicates. Average the readings of the three Total SH reaction replicates to determine the $(A_{324})_{\text{Total SH}}$.

7. Clean the cuvette and read the A_{324} of the first sample tube $(A_{324})_{\text{initial}}$. Add 10 µL of the 50× 4,4′-DTDP Stock Solution to the cuvette and mix well. Incubate the sample for 2 minutes and then determine the $(A_{324})_{\text{final}}$. Clean the cuvette and repeat this step with the next two sample replicates.

Results

Calculations

Calculate the number of maleimide groups for each sample:

1. Calculate the change in absorbance

$$\Delta A_{324} = (A_{324})_{\text{total SH}} - [(A_{324})_{\text{final}} - (A_{324})_{\text{initial}}]$$

2. Calculate amount of maleimide

$$\frac{\text{Moles of Maleimide}}{\text{Mole of Conjugate}} = \left(\frac{\Delta A_{324}}{19{,}800\ M^{-1}cm^{-1}}\right) \times \frac{V}{(S)/(MW\ of\ Sample)}$$

where:
19,800 $M^{-1}cm^{-1}$ = DTDP extinction coefficient at 324 nm
V = Sample Volume (Liter)
S = Weight of conjugate sample (mg)
MW of sample = molecular weight of conjugate

KH,LS,MAM 04/19-1