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)** 1 vl
  Catalog Number **MAK162A**

- **4,4′-DTDP (4,4′-Dithiodipyridine)** 1 vl
  Catalog Number **MAK162B**

- **Assay Buffer** 50 mL
  Catalog Number **MAK162C**

- **DMSO** 1 mL
  Catalog Number **MAK162D**

**Reagents and Equipment Required but Not Provided.**
- Cuvettes or 96 well plate - It is recommended to use clear plates for colorimetric assays.
- Spectrophotometer 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 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>500× MEA Stock Solution</td>
<td>2 µL</td>
</tr>
<tr>
<td>ultrapure water</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

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 ($A_{324}$)_{Total SH}.

7. Clean the cuvette and read the $A_{324}$ of the first sample tube ($A_{324}$)_{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 ($A_{324}$)_{final}. Clean the cuvette and repeat this step with the next two sample replicates.

Note: Samples can also be analyzed in a 96 well plate and analyzed on a spectrophotometric plate reader.

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

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

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

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