

1. PRODUCT AND COMPANY INFORMATION

1.1 Product Identifiers

Product Names: ZW800-1 Maleimide
Product Numbers: 1 mg: 97-02-01-0043
10 mg: 97-02-01-0044

CAS-No.: N/A

1.2 Product Information

Maleimide derivatives permit one-step conjugation of near-infrared fluorophores to thiol groups under mild conditions, in both aqueous and non-aqueous environments. Curadel's maleimide derivatives are of particularly high purity and quality.

1.3 Details of the supplier for the Instructions for Use

Company: Curadel ResVet Imaging, LLC
377 Plantation Street
Worcester, MA 01605
USA
Telephone: 774-243-9515
Fax: 774-243-9517
E-mail: support@resvet.curadel.com
URL: www.curadelresvetimaging.com

1.4 Emergency Telephone Number

Emergency Phone #: 774-243-9515

2. HANDLING

Precautions This contrast agent is intended for laboratory research use only. Not for diagnostic procedures. Not for veterinary or human use.

Wear appropriate protective equipment including laboratory coat, gloves, and eyewear. Avoid contact with skin, eyes, and mouth. Contrast agent will stain clothing and skin.

Preparation Prepare a stock solution of ZW800-1 Maleimide in anhydrous DMSO at a concentration of 10 mM.

Alternative: a stock solution of ZW800-1 Maleimide can be prepared in water, or an aqueous based buffer at a concentration of 1 mg/mL.

Sterile Use: Whenever sterile use is intended, for example, intravenous injection into a research animal or addition to cell culture medium, filter the final NIR fluorophore solution through a 0.22 µm filter.

Storage Lyophilized powder can be stored at room temperature up to one year. Protect from light. Stock solutions should be kept at 4-8 C when in DMSO. Stock solutions in water should be kept at -20 C. Avoid excessive freeze/thaw cycles by aliquoting solution prior to freezing.

Discard stock solutions after 3 months when stored properly.
Discard stock solutions after 1 week when stored at room temperature.

3. USAGE

Small Molecule Conjugation

Prepare a stock solution of ZW800-1 Maleimide as described in the preparation section above. Add to the desired small molecule with a free thiol in a ratio between 1.0-1.1 molar equivalents of the small molecule to ZW800-1 Maleimide dissolved in an aqueous buffer (25-50 mM MES buffer is recommended). Adjust the pH of the reaction to between 6.5-7.0 pH units. Do not exceed a pH of 7.5 for the reaction to avoid hydrolysis of the maleimide. The lower the pH, the slower the reaction will proceed. Reactions at pH 5.5, for example, often require 8 hours or more of reaction time at room temperature. Most maleimide conjugation reactions should be performed at pH 6.5 at room temperature, resulting in completion within 5-15 minutes. If the reaction ceases to progress, check the pH and adjust as necessary with sodium hydroxide or hydrochloric acid. If the reaction still does not go to completion, add an additional half equivalent of the small molecule.

Alternatively, maleimide conjugation can be performed in DMSO using 1-1.1 equivalents of ZW800-1 Maleimide to the small molecule to be labeled. No pH modifiers should be necessary, and the reaction should go to completion at room temperature. The ratio can be adjusted if the desire is to have ZW800-1 Maleimide as the limiting reagent. It is highly recommended not to exceed 1.2 equivalents of ZW800-1 Maleimide. The easiest way to monitor the reaction is to use a liquid chromatographic system (equipped with C18 column) with PDA detector, monitoring at 770 nm.

Large Molecule Conjugation

Prepare a stock solution of ZW800-1 Maleimide as described in the preparation section above. Prepare a solution of the desired large molecule in an aqueous buffer (25-50 mM MES buffer is recommended, 1X PBS is also suitable if required). Adjust the pH, using sodium hydroxide or hydrochloric acid to achieve a pH between 6.5-7.5. Do not exceed a pH of 7.5. The lower the pH for the reaction, the slower the rate of reaction. For example, labeling Cys34 on albumin at pH 5.5 requires an overnight reaction at room temperature to go to completion. ZW800-1 Maleimide has been observed to be stable overnight at room temperature at a pH of 5.5. However, to avoid protein degradation over time it is desirable to have as fast of a reaction as possible. This is best achieved between pH 6.5-7.5 where the reaction time is 15-30 minutes on average. A ratio between 1.0-1.2 molar equivalents of ZW800-1 Maleimide should be used to simplify the cleanup of the reaction. Please note that these ratios should be based on the number of free thiols (free cysteine) available for labeling. Let this react at room temperature for 0.25-2 hours with constant agitation. If the reaction ceases to progress, add an additional equivalent of ZW800-1 Maleimide. Continue this until the reaction is complete. If the reaction still does not progress confirm the pH is still between 6.5-7.5 and adjust as necessary. The easiest way to monitor the reaction is to use a liquid chromatographic system (equipped for size exclusion chromatography) with PDA detector, monitoring at 770 nm. Purification can be easily done using gel filtration chromatography or molecular weight cut off spin columns.