

## 1. PRODUCT AND COMPANY INFORMATION

### 1.1 Product Identifiers

Product Names: ZW700-1 Forte NHS  
Product Numbers: 1 mg: 97-02-01-0009;  
10 mg: 97-02-01-0010  
CAS-No.: N/A

### 1.2 Product Information

N-Hydroxysuccinimide (NHS or –OSu) activated esters are commonly used in bio-conjugation protocols because they are so versatile and easy to use. Activation of the carboxylic acid enables the formation of a stable covalent amide bond to form, making these reagents ideal for one-step conjugation to primary amino groups of small molecules, peptides, antibodies, proteins, and polymers.

Labeling the amino groups of small molecules, peptides, and peptidomimetics using an NHS ester is typically performed in mildly basic anhydrous solvent while at room temperature. However, the labeling of biomolecules needs to be performed in an aqueous environment, in which case hydroxyl ions from water can act as a nucleophile. In these cases, the amount of NHS activated starting material and the pH of the environment is important for proper labeling.

In addition to pH, and assuming that the buffer used does not contain any extraneous primary amines (aside: never use Tris buffer for an NHS labeling), the most critical parameter is the rate of the reaction. Just like in any chemical reaction, the rate (corresponding to completeness of the reaction for a given length of time) is proportional to the product of the two reactants, in this case the primary amine and the NHS ester. In general, approximately 1 mM of each reactant will lead to completion of the reaction. And, the higher the concentration of each the better, with the upper limit only dependent on solubility of the reactants in the chosen buffer. Remember, too, that the concentration of available epsilon amines in a protein depends on how many lysines are present. For example, a protein with 10 lysines used at a concentration of 100  $\mu$ M is actually 1 mM in primary amine, and thus will usually drive the reaction to completion (assuming all lysines are surface accessible).

### 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](mailto:support@resvet.curadel.com)  
URL: [www.curadelresvetimaging.com](http://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 ZW700-1 Forte NHS in anhydrous DMSO at a concentration of up to 150 mM.

**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 ZW700-1 Forte NHS as described in the preparation section above. Add to the desired small molecule with a free amine in a ratio between 1.5-2 molar equivalents of the small molecule to ZW700-1 Forte NHS dissolved in anhydrous DMSO. Add between 3-10 equivalents of N,N-Diisopropylethylamine (exact amount required will depend upon the pKa of the small molecule, other bases can be used as required but it is best to ensure any amine containing bases act as poor nucleophiles) to the reaction mixture and let this react at room temperature for 0.5-2 hours with constant agitation. If the reaction ceases to progress, add an additional equivalent of base and monitor after 15 minutes. Continue this until the reaction is complete. If the reaction still does not go to completion, add an additional equivalent of the small molecule. The easiest way to monitor the reaction is to use a liquid chromatographic system (equipped with C18 column) with PDA detector, monitoring at 660 nm.

**Large Molecule Conjugation**

Prepare a stock solution of ZW700-1 Forte NHS as described in the preparation section above. Add to the desired large molecule (prepared in a buffered aqueous solution) a ratio between 1.5-2 molar equivalents of ZW700-1 Forte NHS. Add the required amount 1M sodium hydroxide in water to the reaction mixture to achieve a pH of approximately 8. Let this react at room temperature for 0.5-2 hours with constant agitation. If the reaction ceases to progress, add an additional equivalent of ZW700-1 Forte NHS. Continue this until the reaction is complete. If the reaction still does not progress confirm the pH is still 8 or greater 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 660 nm.