

4. Measure the absorbance of each solution at 500 nm. Subtract the absorbance value of solution 1 from that of solutions 2–5.
5. Solutions 2–4 should be a pale red. Determine the average absorbance value for solutions 2–4. (The absolute absorbance values may vary depending upon the age and quality of the  $\text{Fe}(\text{NO}_3)_3$ ).
6. Solution 5 should be pale yellow to colorless. Presence of a red color indicates hydrolysis of NHS-biotin in the NHS-biotin reagent. If the absorbance of solution 5 is greater than 10% of the average absorbance of solutions 2–4, the NHS-Biotin reagent is suspect.

#### Ordering Information

Catalog #	Description
170-6529	NHS-Biotin, 4 ml
170-6528	Avidin-HRP, 2 ml
170-6533	Avidin-AP, 1 ml
170-6512	Biotin-Blot Total Protein Detection Kit

CAS #68-12-2 (DMF)

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**Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547 USA**

**LIT424 Rev B**

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# NHS-Biotin

Catalog #  
170-6529

**BIO-RAD**

## Specifications

<b>Contents</b>	4 ml
<b>Product Description</b>	A 75 mM solution of N-hydroxysuccinimide ester of biotin in dimethylformamide (DMF). Molarity is determined by HPLC. The solution contains 5 Å molecular sieves to scavenge water from the solution.
<b>Storage</b>	Store desiccated at -20°C. Cap tightly to prevent water condensation.
<b>Shelf life</b>	1 year at -20°C.
<b>Use</b>	Remove solution through rubber stopper with syringe. Leave the aluminum seal on the bottle.

### **For Biotin-Blot™ protein detection:**

Add 200 µl of NHS-biotin to 100 ml of 0.05 M borate, 0.2% Tween 20. The pH will be between 9 and 9.5. If boric acid is

used, adjust the pH to between 9 and 9.5 with NaOH. Incubate for 15 min with constant agitation. The biotin solution must be prepared immediately before use, as the biotin reagent is completely hydrolyzed in 15 min in borate, Tween solutions. See Bio-Rad's Biotin-Blot protein detection kit instruction manual (catalog #170-6512) for complete instructions.

**Warning** Toxic, contains dimethylformamide. Combustible. Refer to MSDS.

**Note:** Do not use NHS-biotin in buffers containing free amine groups (i.e., Tris, glycine), because they will deactivate the reagent.

## Activity Test for NHS-Biotin

Reagents	0.1 N HCl
required:	1.0 N HCl
	0.05 M Fe(NO <sub>3</sub> ) <sub>3</sub> in 0.5 N HCl
	0.1 N NaOH
	Dimethylformamide (DMF)

1. Prepare 5 test tubes containing the following solutions:  
Solution 1: 1.0 ml of 0.1 N NaOH + 10 µl DMF  
Solution 2: 1.0 ml of 0.1 N NaOH + 10 µl NHS-biotin reagent  
Solution 3: same as 2  
Solution 4: same as 2  
Solution 5: 1.0 ml of 0.1 N HCl + 10 µl NHS-biotin reagent
2. Heat solutions 1-4 to 100°C for 10 min. Do not heat solution 5.
3. Add 0.1 ml of 1.0 N HCl and 100 µl of 0.05 M Fe(NO<sub>3</sub>)<sub>3</sub> in 0.5 N HCl to solutions 1-5. Mix thoroughly.