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Technical Support
Information Bulletin 1198

Resin Loading Measurement by Fmoc Cleavage

Resin loading or substitution is the number of reactive sites per gram of resin and it is typically expressed in units of mmoles per gram of resin. This measurement is important for calculating the amounts of amino acids and reagents required for a solid phase peptide synthesis as well as determining the percent yield of the synthesis procedure. Resin loading can be determined by several methods, including weight change of the resin, elemental analysis and photometric methods. For resins such as Fmoc amino acid loaded resins or Rink Amide resins, one of the easiest and most accurate methods is to photometrically measure the amount of Fmoc released for an accurately weighted sample.

Photometric Measurement of Resin Loading

1. Accurately weigh 10 mg of resin into an eppendorf tube.
2. Add 800 µL of N,N-dimethylformamide (DMF) and allow the resin to swell for 15 minutes.
3. Add 200 µL piperidine.
4. Vortex to ensure good mixing, then let it stand at room temperature for 15 minutes.
5. Transfer 100 µL of the solution to a 1cm quartz cuvette.
6. Dilute the sample with 900 µL of DMF.
7. Measure the absorbance at 301 nm versus a DMF blank.

The general formula for calculating the resin loading is:

$$L = (A_{301} \times V \times d)/(E_c \times w \times M)$$

where

L = Resin loading
A_{301} = Absorbance at 301 nm
V = Volume of the cleavage solution = 1 mL
d = Dilution = 10
E_c = Extinction coeffiient = 7800 mL/mmol*cm
w = Width of the cuvette = 1 cm
M = Weight of the resin sample

Substituting the extinction coeffient, volume, dilution and cell width into the general formula results in this formula which can be used to calculate the resin loading.

$$L = (10 \times A301)/(7800 \times M) \text{ in units of mmols/gram.}$$