# Quick Guide to Biotage® Initiator+ Alstra™

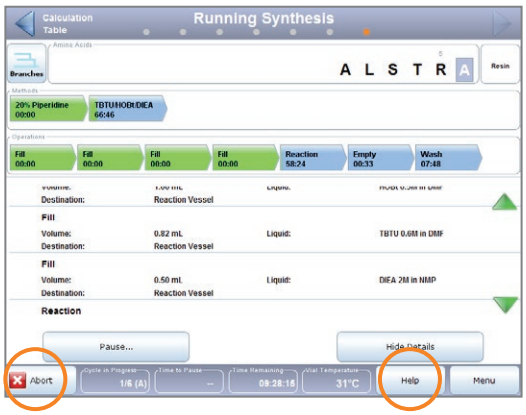
## Navigating the Biotage® Initiator+ Alstra™ Software

Experiments are set up by using the synthesis wizard. To access the wizard, press *Peptide Synthesis* in the main menu or press *Menu* in the bottom pane of the display and then select *Synthesis* in the appearing menu.

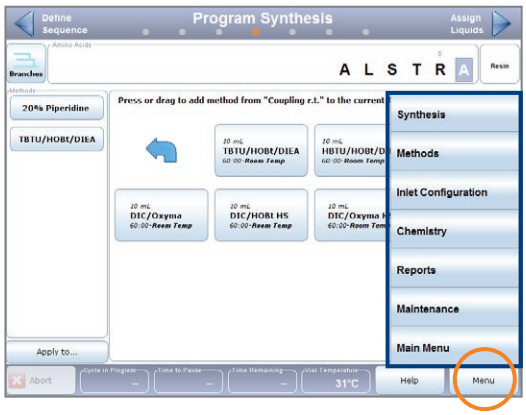
Navigate through the seven steps of the wizard by pressing ▶ (next) or ◀ (previous), or by pressing the bullets in the top pane. Each bullet represents a step in the wizard.

The bottom pane of the display contains information fields for monitoring of reactions and an *Abort* button. It also contains buttons for accessing the online *Help* and a *Menu* where you can enter other parameters (define chemicals and methods, etc.) and then return to the synthesis wizard.


The peptide sequence is defined in the third step of the synthesis wizard.

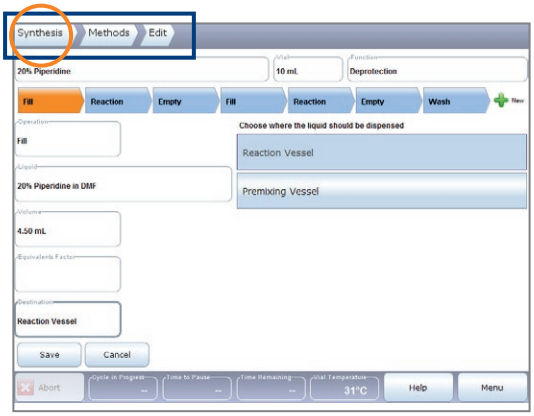




Bottom pane with reaction monitoring fields and buttons.




Menu options.

When defining methods and chemicals, viewing reports, or performing maintenance tasks (*Menu* options), navigation is performed by pressing the desired view in the navigation path in the top pane. The view displayed is the rightmost. To return to the synthesis wizard, press *Synthesis* in the navigation path.




The navigation path displayed when editing a method in the menu option Methods.

**Tip:** In the wizard's Program Synthesis view, methods can only be edited for the current sequence. However, you can create custom methods (that are saved) via the menu option Methods. Saved methods will appear as options in the Program Synthesis view.

To create and save custom methods, refer to Methods View or Instructions/Manage Methods and Folders in the system's online help.



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## Preparing Reagents for Peptide Synthesis

All reagents and amino acids need to be dissolved before use on the Initiator+ Alstra system. Although we do not have a standard concentration, it is recommended to select the concentrations so that a minimum volume of 100 µL is aspirated and dispensed by the robot.

For amino acids, a concentration of 0.2–0.7 M is usually recommended for this system. In predefined Biotage methods, the recommended concentration is 0.2 M for the 5 ml vial, 0.5 M for the 10 ml vial, and 0.7 M for the 30 ml vial. At the higher concentration, the amino acid should be dissolved in NMP. Vigorous stirring may be needed to obtain complete dissolution. In cases where an amino acid is difficult to dissolve, a small addition of DMSO or NMP to the dry powder before adding the main solvent can be beneficial.

**Note:** There is a risk of oxidation of methionine if DMSO is used.

Scale is dependent on resin loading and type. When setting up a synthesis, two important aspects need to be considered:

- The overall volume
- The resin to solvent ratio

The minimum volumes for the vials (see Table 1) are important as they are the minimum volumes for efficient microwave heating and accurate temperature measurements. Do not use microwave heating if the minimum volumes are not reached. The maximum volumes are set to prevent spillage and to obtain efficient washing. All recommendations are based on pre-swollen resin.

The resin to solvent ratio is dependent on the resin type. We recommend that only resins with a bead size in the range of 100 to 200 mesh are used. It is very difficult to predict accurate solvation for all resins. The 5 mL reaction vial needs a higher solvent to resin ratio. As different resin and solvent combinations have different solvation characteristics, the user should manually check that the resin is adequately solvated by the volume that is dispensed by the planned methods, for the particular type of resin and solvent employed, before setting up the synthesis.

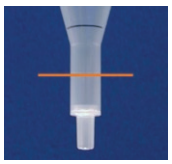
Example: If your method dispenses a total of 3 mL liquid, take the planned amount of resin (e.g. 0.2 g) and let it swell in the solvent of your choice so that it is properly solvated, empty the reaction vial of all extra liquid, and add the 3 mL of the solvent that is closest to the conditions of the method. Ensure that the resin–solvent mixture moves freely and that it is between the minimum and maximum volumes of the vial; please refer to Table 1 for guidelines.

| Vial Size | Scale (mmol) | Possible/Recommended Volume (resin + liquid) |
|---|---|---|
| 5 mL | PEG based: 0.005–0.010<br>Polystyrene: 0.005–0.040 | 0.6–3.5 mL/0.6–1.2 mL |
| 10 mL | PEG based: 0.05–0.40<br>Polystyrene: 0.05–1.00* | 3.5–10.0 mL/3.5–10.0 mL |
| 30 mL | PEG based: 0.50–0.75<br>Polystyrene: 0.5–2.0† | 4.5–20.0 mL/10.0–20.0 mL |

* For UV monitored Fmoc deprotections the scale is 0.05–0.5.
† For UV monitored Fmoc deprotections the scale is 0.5–1.0.

**Table 1.** Vial selection and scale.

**Note:** In order to obtain efficient mixing for the 5 mL vial, it is important to only fill the narrow part of the vial to about half way with resin (in solvated form) and the remainder with solvent. See the image to the right.



## Potential Side Reactions

As solid-phase peptide synthesis (SPPS) at elevated temperatures can enhance some side reactions of certain residues and sequences, care must be taken when synthesizing peptides containing these residues and sequences. Below is a list of known side reactions that may occur for certain residues, as well as some recommendations to circumvent side reactions:

- Epimerization of His residue – couple His derivatives at room temperature or below 50°C. Coupling with a weaker base or non-basic couplings may also help to suppress the epimerization.
- Epimerization of Cys residue – avoid preactivation and use base free activation. Couple at room temperature or below 50°C.
- δ-lactamisation of Arg residue – couple at room temperature or below 50°C.
- Aspartimide formation – use 5% piperazine as deprotection base after incorporation of Asp-XX residue (XX = Gly, Asn, Ser, Thr, Gln, Arg (Pmc)). Longer deprotection times may be needed as piperazine is a weaker base than piperidine.
- Diketopiperazine formation on the second coupling when using C-terminal proline and a resin with an ester linker – use a trityl-based resin.

**Note:** We do not recommend the use of microwave heating during the Fmoc deprotection step due to the potential risk of promoting side reactions such as aspartimide formation and epimerization. All deprotections should be performed at room temperature.

**Part Number: 356231-E**