Rewriting KingFisher 2.6 Protocols in BindIt for KingFisher Software

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Technical Notes

Introduction

Many customers have older KingFisher instruments that shipped with the now-discontinued software KingFisher 2.6. Because of the ease of use and additional features of the current Thermo Scientific™ BindIt software for KingFisher software, or even due to simple incompatibility of the old software with current PC operating systems, people wish to convert their older protocols to BindIt software format so that edits may be made. This tech note explains how to re-write KingFisher 2.6 protocols as BindIt software protocols.

Figure 1. The current high-throughput model, KingFisher Flex

Background

The Thermo Scientific™ Kingfisher™ 96 system was the first model to process samples in full plates of 96. KingFisher 96 could be integrated with robotics for those labs needing to perform nucleic acid or protein extractions in high-throughput formats using KingFisher 96 deepwell plates, KingFisher 96 standard-height plates, and fully skirted 96-well PCR plates. There are still many KingFisher 96 units in use today. The instrument utilized KingFisher Software 2.6, which featured 13 different speed settings. The internal firmware and hardware were set up and calibrated for all 13 settings. The speed selections in the old software include: very slow, slow, medium, fast, very fast, super fast, bottom very slow, bottom slow, bottom medium, bottom fast, grind mix, half mix, and fast dual mix.

Figure 2. The KingFisher 96 (discontinued model), predecessor to the KingFisher Flex

In 2008, we replaced the KingFisher 96 system with a second-generation high-throughput instrument, Thermo Scientific™ Kingfisher™ Flex magnetic particle processor. The Flex is capable of all three formats of the KingFisher 96, plus one more: a 24 deepwell format used for processing volumes of five times that of the largest 96-well format.

Along with the Flex, a new, more intuitive software was developed with greater usability and expanded features. BindIt incorporates the major elements of KingFisher Software 2.6 in that the user enters the liquid volumes into a plate layout and connects the plates to specific steps in the protocol steplist. However, the mix speeds were changed in BindIt because of the internal differences in the KingFisher Flex vs. the KingFisher 96. Additionally, it was found that the high number of speed selections in the older software were not only unnecessary for optimal processing of most kits, but were often confusing to users, who sometimes had trouble deciding which setting to choose for a given step. BindIt features only five mix speeds: slow, medium, fast, bottom, and half mix.

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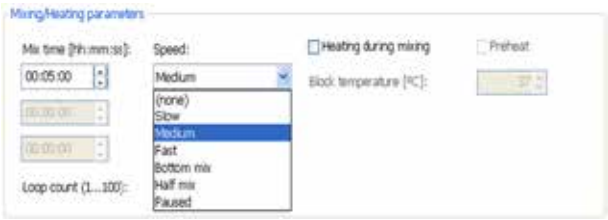

Figure 3. BindIt software offers five mix speeds vs. the KingFisher 2.6 software's 13 mix speeds.

Our applications lab performed testing to determine which speed settings in BindIt most closely align with settings in KingFisher Software 2.6, and created the speed conversion table (Figure 4.) These guidelines should be used regardless of which older model KingFisher is in use – KingFisher 96, KingFisher, or KingFisher mL.

Please note that BindIt can run KingFisher Software 2.6 files (extension .kf2) just as they are, without any problem. However, should the user need to make changes to the protocol, a .kf2 file cannot be edited in BindIt software. Therefore, the following guidelines should be used whenever an editable protocol is required. These instructions describe how to use a KingFisher Software 2.6 protocol as a basis for writing a BindIt protocol.

Using a .kf2 Protocol to Create a BindIt Protocol: Speed Setting Conversion

If the protocol will be run on the same model KingFisher, then the .kf2 file may be imported into BindIt software (Home Tab → Import), opened, and then Saved As with a unique name. This will convert the .kf2 file into an editable BindIt protocol. The speed settings will all change to Medium, so the user should follow the guidelines in the chart and change the speeds appropriately, depending on what they were in the KingFisher 2.6 software. For example, this would be the preference when converting a .kf2 file that was run on a KingFisher 96 into a BindIt protocol to be run on another KingFisher 96. .kf2 files that were written for KingFisher 96 cannot be run on a KingFisher Flex, regardless of whether they have been converted into a BindIt protocol.

To write a protocol for a different instrument – for example, a protocol being used on a KingFisher 96 that now needs to be run on a KingFisher Flex – the user should first open KingFisher 2.6 software and either keep the protocol open, or export it as an html document, for use as a reference. To export, click Protocol → Export Printout → (select desired protocol from database windows) → Save → (select destination) → Save. This creates an html file. Double-click the file icon to open the file in Windows Explorer. To print, select File → Print.

Next, open BindIt for KingFisher software. Click New and select the appropriate KingFisher model from the pull-down menu. ***Note**: If an incorrect model is selected, the protocol will not run on the intended instrument*. For ease of use, the icons have been color coded.

KingFisher (part number 5400000)

KingFisher mL (part number 5400050)

KingFisher 96 (part number 5400500)

Enter the information on the Layout and Protocol tabs and connect each step with an appropriate plate as usual. For assistance in learning to use BindIt software, click on the Help button at the top of the window or resource Thermo Scientific Applications Support for KingFisher Products.

When ready to program the mixing speeds, use the following guidelines.

If KF2.6 uses…	…then select this speed in BindIt.
Very slow or slow	Slow
Medium Fast Bottom very slow Bottom slow	Medium
Very fast or superfast	Fast
Half mix	Half mix
Grind mix Bottom medium Bottom fast	Bottom mix

Figure 4. Speed conversion table

The Fast Dual mix in the KingFisher software has no selectable option in BindIt, but may be programmed as follows:

1. In the right-pane window of the Mix step, select "Show Advanced."
2. Under Mixing/Heating parameters, enter "Fast" for 10 seconds in the first Mix Time field and "Bottom Mix" for 10 seconds in the second Mix Time field.
3. Divide the total Fast Dual time in the original protocol by 20 seconds.
4. Enter this number in the Loop Count field.

This programs a step that is equivalent to the Fast Dual mix.

Using a .kf2 Protocol to Create a BindIt Protocol: Collection Setting Conversion

In the KingFisher 2.6 software, the user is asked to enter the number of times the magnetic rods go into the wells to collect the beads in order to move them to the next step's plate or well. The default value is three, and the maximum is 100, with three to 10 being typical values.

In BindIt software, bead collection is broken down into two separate components, Collect Count and Collect Time. Collect Count is the number of times the magnet goes into the wells, and has a maximum of 5. Collect Time is defined as the amount of time the magnetic tips stay at the bottom of the wells, and can be entered from 1 to 30 seconds. Because the rods are only magnetic at the tips, the magnet will move very slowly through the liquid column in order to ensure all beads are given enough time to be attracted to the tips.

Using the default parameters of Collect Count = 3 and Collect Time = 1 second does not mean that the beads will be collected in three seconds. The amount of time for the magnets to collect will depend not only on Collect Count and Collect Time, but also on format, plate type, and how much liquid the user entered into the plate layout for that step. Therefore, converting the Collect parameter from KingFisher 2.6 exactly into the same number of counts and time in BindIt is not possible. A higher number in the .kf2 file would indicate more Collect Counts and/or a longer Collect Time in BindIt.

Generally speaking, the default BindIt parameters of Collect Count = 3 and Collect Time = 1 are sufficient for most kits' beads during the Wash and Elution steps, where the surrounding liquid is relatively clean. For the Bind Step, it is recommended to increase Collect Count and/or Collect Time if the sample is "dirty," e.g., soil, stool or tissue lysates, or for samples that may contain a significant amount of debris or extraneous biochemicals and macromolecules. Using a Collect Count of 5 and Collect Time of 10 seconds is usually sufficient for dirty samples, but erring on the side of conservatism and using a Collect Time = 30 will do nothing more than extend the time of the protocol, and ensure that all beads are collected. The first aim is to achieve optimal yield and purity. Collect Count and Collect Time may then be pared back in order to minimize the protocol time.

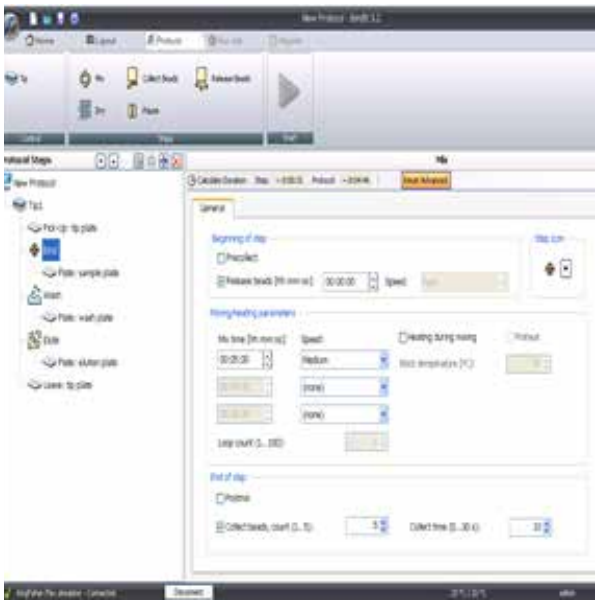

Figure 5. Generally, it is advisable to increase the Collect Count and Collect Time for Bind Steps.

Note that in KingFisher 2.6 software, the user is prompted to designate where eluted beads are to be discarded ("Release Beads.") In BindIt software, the beads will be discarded along with the tip comb unless a separate, optional Release Beads step is added. For KingFisher Flex and KingFisher Duo, the added Release Beads is not necessary. For KingFisher (5400000) and KingFisher mL (5400050), it is recommended to add the Release Beads at the end since the tip comb is not discarded, but remains on the magnetic rods until manually removed.

Summary

In summary, it is always possible to convert .kf2 protocols into BindIt protocols and still achieve excellent yield and purity of the extracted target. Minor optimization may be required due to the differences in mix speeds and bead collection parameters, or to minimize protocol times.

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