

Improve Nucleic Acid Extraction using an Adjustable Tip Spacing Pipette

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Key Words

Nucleic acid extraction, magnetic bead, ClipTip, electronic pipette, KingFisher

Goal

Thermo Scientific™ KingFisher™ Duo Prime automated purification system is a fast way to extract high quality nucleic acids in low- to medium-throughput labs. With Thermo Scientific™ E1-ClipTip™ Adjustable Tip Spacing Equalizer Multichannel Electronic Pipette it is possible to enhance the extraction process by using only one tool to do all of the pipetting. Adding samples, reagents and magnetic beads on the deep-well 96-plate or 12-well elution strip can be done entirely using one pipette. The E1-ClipTip Equalizer 6-channel, 15-1250 µl pipette will save hands-on time and reduce repetitive motions required in the process.

Introduction

The samples for DNA extraction with KingFisher Duo Prime may vary from plant leaves to mouse ears, or any organism of interest. The sample can be extracted using a variety of sample vessels available; e.g. blood tubes of different sizes, 15ml/50ml conical tubes, microcentrifuge tubes or microplate format.

KingFisher Duo Prime works in standard 24- and 96-plate formats and typically there is a need to use several pipettes of different volume settings to fill the reagents on the plate and the elution strip. Not anymore! Thermo Scientific KingFisher deep-well 96-plate and KingFisher Duo Prime elution strips can easily be filled using the E1-ClipTip Equalizer pipette. The adjustable tip spacing feature makes it possible to transfer samples from tubes to plates, and dispense magnetic particles and buffers effortlessly with multi-dispensing pipetting function.

It is also easy to program, save and name a ready-to-use protocol to fill a plate for routine applications in the Programs mode.



E1-ClipTip pipettes feature a unique Matrix function that utilizes step-based programming which allows creation of fully customized pipetting protocols. This is an excellent option when a variety of pipetting functions are required in a unique order to complement different research protocols.

In this technical note, the simplicity of using E1-ClipTip Equalizer 6-channel, 15-1250 µl pipette to dispense samples and reagents on the KingFisher Duo Prime is described with an example of DNA extraction from human cells. Use one pipette for all sample and reagent steps with easy access to useful functions such as mixing and multi-dispensing, to increase pipetting efficiency.

Materials and Methods

The E1-ClipTip Equalizer 6-channel, 15-1250 μ l pipette (#4672090) was programmed for pipetting all samples and reagents for DNA purification from human cells using KingFisher Cell and Tissue DNA Kit (#97030196) on KingFisher Duo Prime (#5400110). 12 parallel samples of 5×10^5 HeLa cells were used for testing. Cell lysis was performed according to kit instructions and the lysed samples were pipetted from microcentrifuge tubes to the KingFisher deep-well 96-plate using a preprogrammed E1-ClipTip Equalizer 6-channel, 15-1250 μ l pipette.

Two programs were generated using the Matrix function. One program was created to guide through the sample row pipetting “KF CT Sample row Duo” and another one for pipetting washing and elution buffers “KF CT Buffers Duo”. (Figure 1).

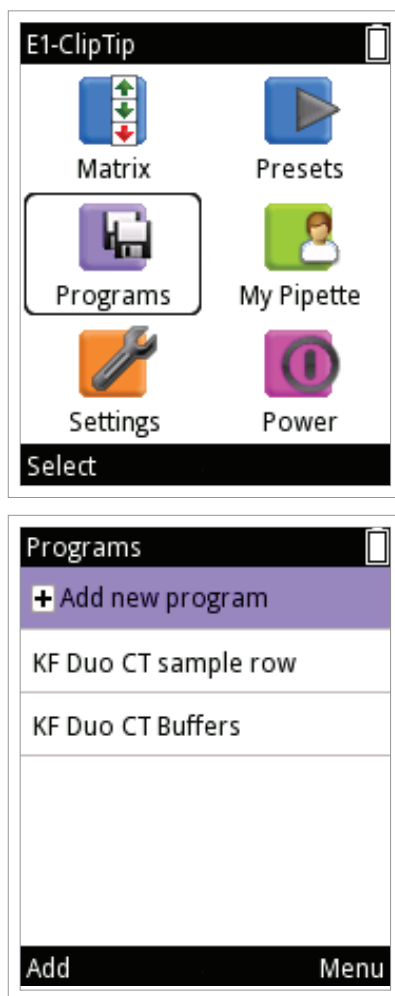


Figure 1. Adding new programs into E1-ClipTip pipette.

Shortcuts can be created for the most commonly used programs on the E1-ClipTip main menu for straightforward and quick access in the laboratory. (Figure 2).



Figure 2. Shortcuts for KingFisher Cell and Tissue DNA Kit and KingFisher Demo pipetting programs on the E1-ClipTip main menu.

KingFisher Cell and Tissue DNA Kit instructions were followed to adjust steps and volumes on E1-ClipTip program according to Table 1. Thermo Scientific ClipTip 1250 pipette tips (#94420813) were used in all pipetting steps. The samples and reagents were pipetted on the KingFisher deep-well 96 plate (#95040450) except for the Elution Buffer, which was pipetted on the KingFisher Duo elution strip (#97003520).

Reagent	Volume, μ l	Vessel	E1-ClipTip pipetting function
KingFisher Magnetic Beads	25	2 ml tube	Mix, Fill 300 μ l, Multi dispense 12 times using single channel
Binding Buffer	360	Reservoir	Fill 720 μ l, Multi dispense 2 times using all channels
Lysed cell sample	225	Tube rack	Fill 225, Dispense 6 samples simultaneously using Equalizer, Repeat
Wash Buffer 1	600	Reservoir	Fill 1200 μ l, Multi dispense 2 times using all channels
Wash Buffer 2	600	Reservoir	Fill 1200 μ l, Multi dispense 2 times using all channels
Wash Buffer 3	800	Reservoir	Fill 800 μ l, Dispense using all channels, Repeat
Elution Buffer	100	Reservoir	Fill 200 μ l, Multi dispense 2 times using all channels

Table 1. E1-ClipTip pipetting function list for dispensing KingFisher Cell and Tissue DNA Kit samples and reagents for KingFisher Duo Prime.

Homogenous solution of magnetic beads is very important to reach uniform results. To ensure evenness of bead distribution, magnetic beads are mixed using the pipette, prior to aspirating the solution for multiple dispense using a single channel of the E1-ClipTip pipette. (Figure 3).



Figure 3. Magnetic beads pipetting step including mixing of the solution.

“KF_TissueDNA_Duo” protocol was started on the KingFisher Duo Prime to perform the DNA purification. The filled deep-well 96-plate and 12-well elution strip were inserted into the instrument following the instructions on the user interface. Thermo Scientific™ Multiskan™ GO microplate spectrophotometer (#51119300) was used to determine DNA quantity and quality by common absorbance measurements.

Results

Genomic DNA purification from HeLa cells resulted even yields of high quality gDNA. The picture from an agarose gel shows clear bands indicating intact gDNA and the absorbance spectra shows extracted gDNA to be pure without remains of proteins or other impurities (Figures 4a and 4b).

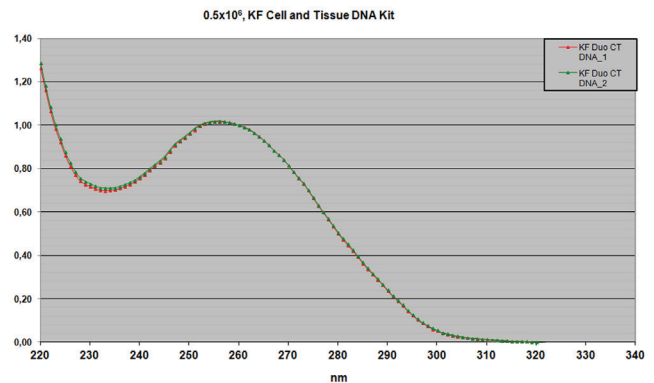
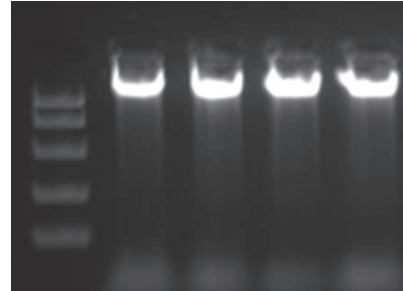


Figure 4. a). Agarose gel and b). DNA spectra (A220 – A320 nm) of gDNA purified from HeLa cells.

Conclusions

The E1-ClipTip Equalizer 6-channel, 15-1250 µl pipette with adjustable tip spacing is an optimal solution for dispensing sample and reagents for KingFisher Duo Prime nucleic acid purification process. By using the E1-ClipTip Equalizer 6-channel pipette, it is possible to use just one pipette and one size of pipette tips to easily fill in reagents for all the steps when dispensing volumes from 25 to 800 µl. The adjustable tip spacing helps to reduce repetitive motions and pipetting time when working between different labware formats.

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