# KingFisher™ Duo Prime Purification in combination with the truXTRAC® cfDNA Kit

Magnetic Bead-based Purification of Circulating Cell-Free DNA (cfDNA) on the KingFisher™ Duo Prime Purification System

Addendum to Product PN 520221

## INTENDED USE

The truXTRAC® cfDNA Kit – Magnetic Bead (PN 520221) is intended for use in research applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

## INTRODUCTION

This protocol is an Addendum to the truXTRAC® cfDNA Kit – Magnetic Bead Purification (Product PN 520221). It describes the semi-automated magnetic bead-based purification of Adaptive Focused Acoustics (AFA)-conditioned plasma on the KingFisher Duo Prime Purification System (Thermo Fisher, PN 5400110).

All reagents necessary for purification of cfDNA on the KingFisher Duo Prime are contained in the truXTRAC® cfDNA Kit – Magnetic Bead (PN 520221).

The protocol enables purification of plasma samples from up to six different donors. For each donor, cfDNA can be purified from 1, 2, 3 or 4 ml of plasma per run.

***Note for first time users:***
Please contact Covaris Application Support (ApplicationSupport@covaris.com) if you have any questions.

## REVISION HISTORY

| Part Number | Revision | Date | Description of change |
|---|---|---|---|
| 010443 | A | 2/18 | Release of truXTRAC cfDNA Kit– Magnetic Bead |
| 022038 | B | 4/20/18 | Corrected buffer name |

## KIT CONTENTS

- Refer to the truXTRAC® cfDNA Kit – Magnetic Bead Purification Manual

**SDS INFORMATION IS AVAILABLE AT** http://covaris.com/resources/safety-data-sheets/

## LABORATORY EQUIPMENT, CHEMICALS AND CONSUMABLES TO BE SUPPLIED BY USER

**Laboratory Equipment**

- KingFisher Duo Prime Purification System (Thermo Fisher, PN 5400110)

**Chemicals**

- Refer to Refer to the truXTRAC® cfDNA Kit – Magnetic Bead Purification Manual

**Consumables**

- KingFisher Duo 6-tip combs (Thermo Fisher, PN 97003510)
- KingFisher 24 deepwell plates (Thermo Fisher, PN 95040470)
- 50 ml conical tubes (e.g., Eppendorf, PN 0030122186)

## cfDNA MAGNETIC BEAD PURIFICATION ON THE KINGFISHER

This protocol starts with AFA-conditioned plasma that was obtained after **Step E in Section-4** in the truXTRAC® cfDNA Kit – Magnetic Bead Purification Manual.

**Important: Reagents must be prepared prior to starting this protocol as described in the truXTRAC® cfDNA Kit – Magnetic Bead Purification Manual Section-2.**

The protocol allows to purify plasma samples from 6 different donors. Per donor, cfDNA from 1, 2, 3 or 4 ml of plasma can be purified per run.

A schematic plate map for the KingFisher Duo Prime Purification System is shown in Figure 1a and 1b.

1. After completing Step E in Section-4 of truXTRAC® cfDNA Kit – Magnetic Bead Purification Manual, open the 2 ml milliTUBE and transfer the AFA-conditioned plasma to a 50 ml conical tube.

**Note: Pool the AFA-conditioned plasma samples from the same donor into the same tube. Conditioned plasma from up to four 1 ml starting aliquots can be pooled.**

2. Add 710 µl Buffer BB2 per 1 ml starting plasma volume (follow instructions in Table 1) and vortex for 10 seconds.
3. Add 1.6 ml of Magnetic Bead Suspension/Isopropanol Mix per 1 ml starting plasma volume (follow instructions in Table 1) and vortex for 10 seconds.

**Important: Steps 2. and 3. must be done sequentially, with thorough mixing after each addition. DO NOT MIX ALL THE COMPONENTS TOGETHER AT THE SAME TIME.**

**Table 1 – Preparation of AFA-Conditioned Plasma for the KingFisher Duo Prime**

| Starting Plasma Volume | 1 ml | 2 ml | 3 ml | 4 ml |
|---|---|---|---|---|
| Buffer BB2 | 0.71 ml | 1.42 ml | 2.16 ml | 2.84 ml |
| Magnetic Bead Suspension/Isopropanol Mix | 1.6 ml | 3.2 ml | 4.8 ml | 6.4 ml |
| KingFisher Protocol File https://..... | 1 ml truXTRAC Protocol.bdz | 2 ml truXTRAC Protocol.bdz | 3 ml truXTRAC Protocol.bdz | 4 ml truXTRAC Protocol.bdz |

4. Load the AFA-conditioned plasma/Buffer BB2/Magnetic Bead mix from the 50ml tube into the Plasma Bind Plate following the lay-out in Figure 1a.
   Load 4.0 ml of the mixture per well (one well will be loaded for each 1 ml of patient sample).

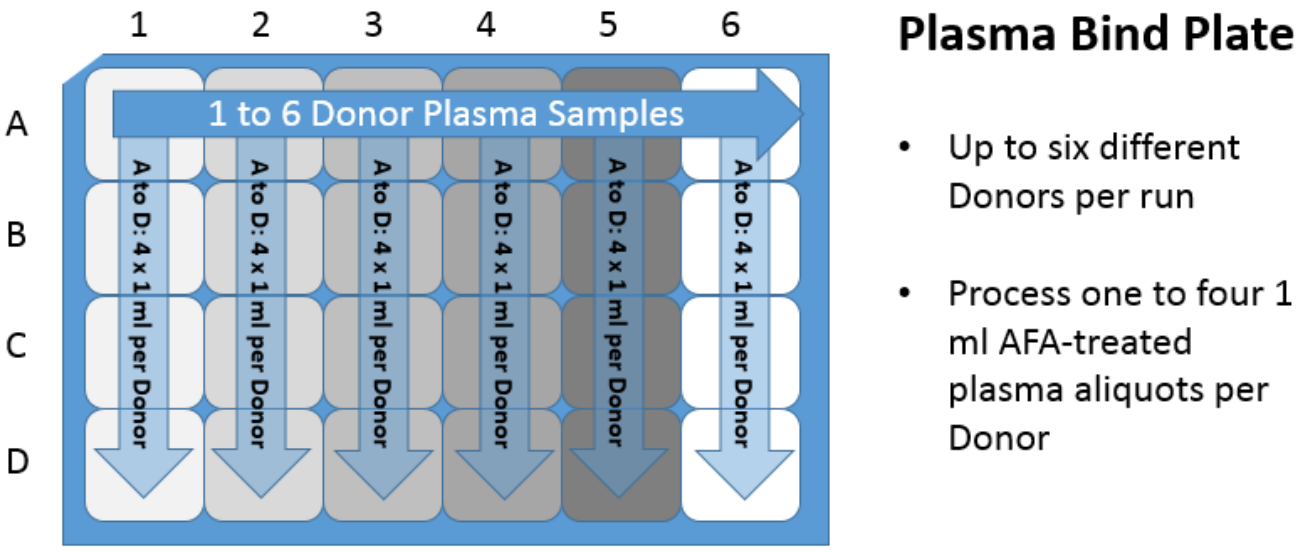


**Figure 1a: Plasma Bind Plate Lay-Out**

5. Set up the Wash + Elution Plate as laid out in Figure 1b.
   a. Add 50 µl to 100 µl of Elution Buffer BE into wells A1 to A6.

**Note: a minimum of 75 µl is required for processing 3 and 4 ml of starting plasma volumes to avoid yield loss.**

   b. Add 1 ml Buffer WB2 into wells D1 to D6.
   c. Add 0.6 ml of 80% EtOH into wells C1 to C6.
   d. Add 0.3 ml of 80% EtOH into wells B1 to B6 and add the 6 Pin Tip Comb into this well row.

**Important: In order to minimize evaporation of the Isopropanol and Ethanol contained in the Wash Reagents, it is highly recommended to prepare this plate last and proceed with the purification on the KingFisher immediately.**

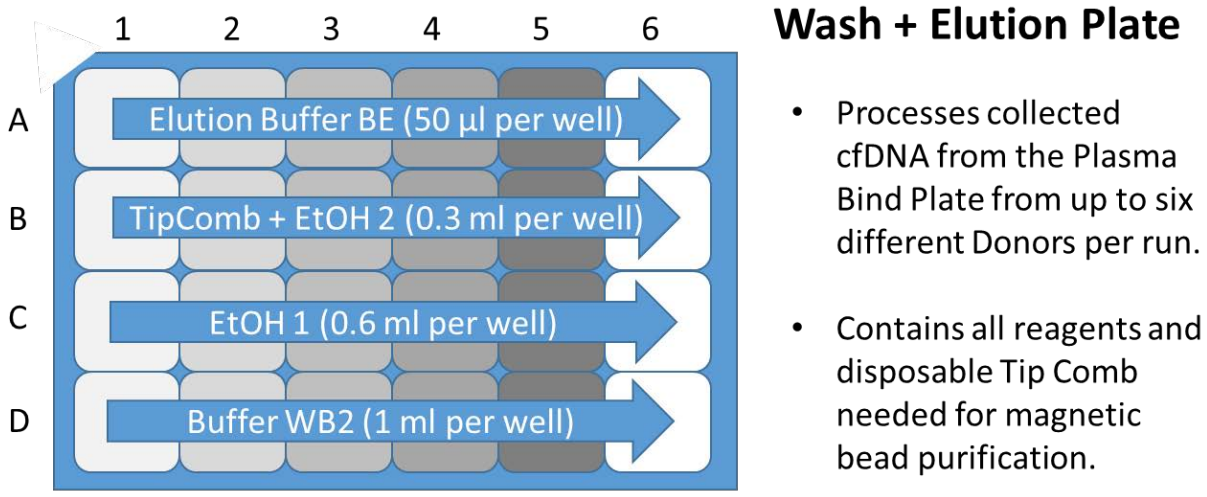


**Figure 1b: Wash + Elution Plate Layout**

6. Turn on the KingFisher Duo Prime and use the Directional Pad to select the appropriate Protocol File as explained in Table 1.

**Important: Do not load the plates before pressing "Play"**

7. Press "Play" and follow the Prompts that guide you through the plate loading procedure and initiation of the purification process.
8. Close the window.
9. After the final Prompt ("Unload Wash-Elution Plate and press the "Check Mark"), unload the Wash + Elution Plate and transfer the eluates in Wells A1 through A6 into 1.7 ml DNA LoBind Eppendorf Tubes. Store purified cfDNA at < -18°C.