Instructions for Isohelix BuccalFix Plus DNA Isolation Kit: BFP-50/BFP-3

Product Details

Isohelix BuccalFix Plus DNA Isolation Kits have been specifically formulated to produce high DNA yield and purity from buccal swabs stabilised in BuccalFix tubes. The kits have been fully optimised at Cell Projects for use on buccal cell samples and offer reduced handling times, increased DNA yields and many other important technical benefits for their use in manual, 96-well or other high throughput formats.

Key Benefits

- ✓ Optimised for buccal cells
- ✓ Fast handling times
- ✓ High purity and yield
- ✓ No solvent based chemicals
- ✓ Protocol integrated to swabs
- ✓ Manual or high throughput formats
- ✓ No columns or filtration
- ✓ Less consumables wastage

Kit Contents

Isohelix BuccalFix Plus DNA Isolation Kit			
Catalogue No.	BFP-50	BFP-3	Storage temperature
Number of preps	50	3	
Proteinase K	1 x 22mg*1	2.2mg*2	4^{0}C after reconstitution
Solution BP (DNA Precipitation buffer)	35ml	2.1ml	Room temperature
Solution TE	5ml	300µl	Room temperature
Solution BLS (Lysis and Stabilisation buffer)	5ml	300µl	Room temperature
DNA Rehydration buffer	5ml	300µl	Room temperature

*1 Reconstitute vial with 1.1ml sterile ddH_2O before first use, store at 4^{0}C after reconstitution.
*2 Reconstitute vial with 110µl sterile ddH_2O before first use, store at 4^{0}C after reconstitution.

Storage

Isohelix BuccalFix DNA Isolation Kits are shipped at ambient temperature.
Please note that on arrival the kit components should be stored according to the table above.
The kits are stable up to the expiry date if stored as instructed. See box label for expiry date.

Equipment and reagents to be supplied by user

- Water bath or heating block at 60^{0}C
- Pipettes with disposable tips
- Microcentrifuge (with rotor for 2 ml tubes)
- 1.5ml microcentrifuge tubes
- Vortexer

Before Starting

1. Prepare a waterbath or heating block at 60^{0}C
2. Reconstitute the Proteinase K by adding the appropriate amount of sterile ddH_2O as shown above.

Safety and Use of the BuccalFix DNA Isolation Kits

The BuccalFix DNA Isolation kits are intended for use by qualified professionals trained in potential laboratory hazards and good laboratory practice. If direct information is not available on any of our compounds this should not be interpreted as an indication of product safety.

This kit has been designed for research use only

Protocol for BuccalFix Plus DNA Isolation Kit for use with BuccalFix Tubes

1. Add 20µl PK solution to the BuccalFix tube containing the buccal swab. Vortex briefly.
2. Incubate the tube in a 60^{0}C water bath for 1 hour, or a minimum of 30 minutes. Vortex briefly.
3. Transfer the liquid in the tube (approx. 400µl) into a 1.5ml centrifuge tube using a sterile pipette tip.
4. Optional step to increase yield:
   Tip the swab head into a sterile 1.5ml centrifuge tube so that the swab head is uppermost. Spin the tube briefly and using a sterile pipette tip add the recovered supernatant to the 400µl collected previously.
5. Add 400µl BP solution to the tube from step 3, **(500µl if using the optional step 4)**. Vortex briefly. The solution may look cloudy at this point.
6. Place the tube in a microcentrifuge (*with hinge positioned outwards so the liquid can be removed from the opposite side*) and spin at maximum speed (13.4K rpm/12,000 x g) for 10 minutes. The pellet will contain both the DNA and other impurities. Note the pellet may be a large white pellet at this point.
7. Pour off the supernatant carefully then re-spin the tube briefly and remove any remaining liquid carefully with a pipette tip taking care not to disturb the DNA pellet. Note it is important to remove all of the liquid
8. Add 100µl TE solution to the tube. This volume may be decreased to as little as 50µl if a higher concentration of DNA is required.
9. Vortex or pipette up and down for 20 seconds or longer to dislodge the pellet from the tube wall and to disperse the white pellet material as fully as possible. Leave for 2 to 5 minutes at room temperature, longer if a reduced volume of TE has been used, then re-vortex for 10 – 20 seconds. If the pellet is large it may not be possible to fully disperse the pellet. Note: The pellet contains insoluble impurities which will be removed in step 10.
10. Re-spin the tube for 15 minutes at maximum speed (13.4K rpm/12,000 x g) to remove the insoluble impurities. Transfer the supernatant containing the DNA to a sterile 1.5ml tube, being careful not to disturb the pellet. Discard the tube with the pellet.
11. **The sample purity can now be assessed by nanodrop. (Yield should be assessed using a fluorometric assay such as a Qubit assay for accuracy.) For most samples the DNA isolation will be completed at this point, however if the sample purity is not sufficient you may continue with the steps below.**
12. Add a volume of BLS solution equal to the sample volume, vortex to mix. Example 1: If your sample volume is 100µl, add 100µl BLS.
13. Add a volume of BP solution equal to the total volume of sample + BLS in step 13. Example 1: Add 200µl BP solution. Vortex to mix.
14. Place the tube in a microcentrifuge (*with hinge positioned outwards so the liquid can be removed from the opposite side*) and spin at maximum speed (13.4K rpm/12,000 x g) for 10 minutes. Remove all liquid carefully with a pipette tip. Note: The DNA pellet may not be visible. If necessary, re-spin the tube briefly and remove any remaining liquid carefully with a pipette tip taking care not to disturb the pellet.
15. Add 100µl DNA rehydration buffer (or less if your sample volume in step 9 was less than 100µl, or you require a more concentrated DNA sample), vortex and stand at room temperature for 2 to 5 minutes to allow the DNA to fully re-hydrate.

The DNA sample is now ready for use in downstream applications such as amplification.

Store the DNA sample at 4^{0}C for short term storage or -20^{0}C for long term storage.
The expected yield from a buccal swab is on average 1 to 10µg DNA (10 to 100ng/µl) from an adult.