XIT™ Genomic DNA from Buccal Cells

For Extraction of Genomic DNA from Buccal/Cheek cells

INTRODUCTION

The *XIT*™ Genomic DNA kit is designed for the isolation of genomic DNA from Buccal cells. The *XIT*™ kit uses the principle of cell lysis, protein digestion and precipitation and finally DNA precipitation to isolate high quality genomic DNA. *XIT*™ Genomic DNA from Buccal cells kit protocol is designed to isolate Buccal cells with a swab or mouthwash method. The purified DNA has an A_{260}/A_{280} ratio between 1.8-2.0 and has yields ranging between 3-5ug/swab depending on the amount of collected tissue.

ITEM(S) SUPPLIED	Cat. # 786-343 *Up to 25 samples*	Cat. # 786-344 *Up to 50 samples*	Cat. # 786-341 *Up to 25 samples*	Cat. # 786-342 *Up to 250 samples*
XIT™ Lysis Buffer	10ml	2 x 10ml	10ml	100ml
LongLife™ Proteinase K	0.5ml	0.5ml	0.5ml	12.5ml
XIT™ Protein Precipitation Buffer	2.5ml	2 x 2.5ml	2.5ml	25ml
TE Buffer	1.5ml	2 x 1.5ml	1.5ml	20ml
LongLife™ RNase	0.5ml	0.5ml	0.5ml	0.5
Cheek Collection Brushes	25	50	-	-

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the *LongLife*™ Proteinase K and *LongLife*™ RNase at -20°C all other kit components at room temperature. The kit components are stable for 1 year, if stored properly.

ITEMS NEEDED BUT NOT SUPPLIED

- Isopropanol
- 70% ethanol
- 0.9% saline solution (9g NaCl in 1L DI water) for mouthwash collection.

PREPARATION BEFORE USE

1. Preheat a water-bath or heating block to 55°C.
2. Equilibrate TE Buffer to 50-60°C.

PROTOCOL FOR COLLECTION BRUSH

1. Use the Cheek Collection Brush to collect your cheek cells by scraping the inside of your cheek. The best place for collecting cells is the area around the gum line. Just gently twirl the brush 10 times at each gum line.
2. Place the brush into a tube containing 400µl *XIT*™ Lysis Buffer and leave in the solution for 10 minutes at room temperature periodically twirling the brush.
3. Remove the brush from the solution, ensuring that you thoroughly scrape the brush on the side of the tube, releasing the collected cells into the *XIT*™ Lysis Buffer. The solution should be slightly cloudy and viscous.
4. Add 10µl *LongLife*™ Proteinase K to the tube, mix tube by inverting 25 times and incubate at 37°C for 1hour. If possible, invert the tube periodically during the incubation.
5. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
6. Centrifuge at 16,000g for 5 minutes. Carefully, transfer the supernatant to a new tube.

NOTE: *The supernatant should be clear. If not, repeat the centrifugation.*

7. Add 400µl isopropanol to the supernatant and mix by gently inverting the sample at least 20-25 times.
8. Centrifuge at 14,000rpm for 5 minutes.
9. Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the pellet.
10. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
11. Centrifuge at 14,000rpm for 5 minutes.
12. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
13. Add 50µl TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).
14. Rehydrate the genomic DNA by incubating at 55-65°C for one hour, followed by an overnight incubation at room temperature to ensure complete genomic DNA hydration.
15. Store the extracted DNA at 4°C, for long term storage store at -20 or -80°C.

PROTOCOL FOR MOUTHWASH

1. For each sample, use 15ml 0.9% saline solution. Vigorously swirl ~5ml 0.9% saline solution around the mouth and deposit into a 50ml conical tube. Repeat the mouthwash twice with the remaining 0.9% saline solution. Deposit mouthwash in the same tube.
2. Centrifuge cells for 5 minutes at 5,000g. Discard the supernatant.
3. Add 1ml 0.9% saline solution to the cell pellet, resuspend and transfer to a 1.5ml microfuge tube. Centrifuge for 2 minutes at ~10,000g. Discard the supernatant.
4. Add 400ul *XIT*™ Lysis Buffer to the cell pellet and mix sample by gently pipetting up and down.
5. Add 10µl *LongLife*™ Proteinase K to the tube, mix tube by inverting 25 times and incubate at 37°C for 1hour. If possible, invert the tube periodically during the incubation.
6. Add 90µl *XIT*™ Protein Precipitation Buffer to the sample and mix by inverting the tube 10-20 times.
7. Centrifuge at 16,000g for 5 minutes. Carefully, transfer the supernatant to a new tube.

   NOTE: *The supernatant should be clear. If not, repeat the centrifugation.*
8. Add 360µl isopropanol to the supernatant and mix by gently inverting the sample at least 20-25 times.
9. Centrifuge at 14,000rpm for 5 minutes.
10. Discard the supernatant and use a pipette to carefully remove remaining liquid without disturbing the pellet.
11. Add 200µl 70% ethanol and invert the tube twice to wash the pellet.
12. Centrifuge at 14,000rpm for 5 minutes.
13. Discard the supernatant and drain the tube on a piece of clean absorbent paper. Allow to air dry for 15 minutes.
14. Add 50µl TE buffer and 1µl *LongLife*™ RNase to remove the RNA (if required).
15. Rehydrate the genomic DNA by incubating at 55-65°C for one hour, followed by an overnight incubation at room temperature to ensure complete genomic DNA hydration.
16. Store the extracted DNA at 4°C, for long term storage store at -20 or -80°C.

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*