Safety information

- Wear appropriate skin and eye protection throughout the extraction procedure
- 5x Enrichment buffer BLL contains 0.1 % sodium azide which is a harmful chemical. In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Lysis buffer BLQ contains high concentrations of salts and detergents. Note: In case of accidental contact, thoroughly rinse or flush the affected areas with water
- Isopropanol and ethanol are volatile and highly flammable. Keep away from naked flames.

Kit component	GHS symbol	Hazard phrases	Precaution phrases
5x Enrichment-buffer BLL	 Danger	H318	P280/P305+P351+P338
Lysis buffer BLQ	-	-	-
Protease	 Danger	H315/H319/ H334/ H335	P261/P305+P351+P338/ P342+P311
Resuspension buffer BL	-	-	-

SDS (Safety data sheet) are available at our “Genomics Resource Center” on our webpage www.lgcgroup.com/genomics.

www.lgcgroup.com/genomics
Email: genomics@lgcgroup.com

Ostendstr. 25 • TGS Haus 8 • 12459 Berlin • Germany
Tel: +49 (0)30 5304 2200 • Fax: +49 (0)30 5304 2201

Units 1 & 2 • Trident Industrial Estate • Pindar Road
Hoddesdon • Herts • EN11 0WZ • UK
Tel: +44 (0)1992 470 757 • Fax: +44 (0)1438 900 670

100 Cummings Center • Suite 420H • Beverly • MA 01915 • USA
Tel: +1 (978) 232 9430 • Fax: +1 (978) 232 9435

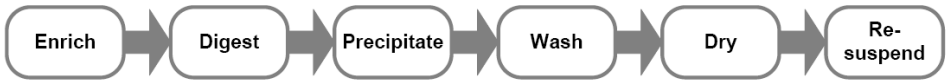
40810/5.00-2016-08

PLUS XL manual kit

Catalogue number **40801** and **40810**
(For research use only. Not for use in diagnostic procedures.)

Description

PLUS XL chemistry has been developed as a cost-effective, easy and fast method for extracting DNA from large volumes of whole blood. The PLUS XL manual kit protocol starts with an enrichment step for the DNA containing cell components. Residual proteins are then digested with a protease and the DNA subsequently precipitated, washed, dried and then re-suspended.



Kit uses

PLUS XL manual kits are used to extract DNA from whole blood. The method was developed and optimised using 10 mL of whole blood with an initial cell enrichment step. The amount of DNA extracted depends on the amount of DNA present in the blood sample (can be affected by the health status of donor). Yields in the region of 400 µg total DNA are achievable from 10 mL of whole blood. The following anticoagulants have been tested and found to be compatible with PLUS XL manual kits:

- EDTA
- Heparin
- Citrate

For additional information or advice on protocols please contact our application specialists via email: info.de@lgcgroup.com or Tel: +49 (0)30 5304 2200.

Kit content

	Colour	Cat. **40801**	Cat. **40810**
5x Enrichment buffer BLL	-	125 mL	375 mL
Lysis buffer BLQ	Blue	100 mL	300 mL
Protease	Grey	4,4 mg	22 mg
Resuspension buffer BL	Black	15 mL	100 mL

Additional required reagents:

- Ultra pure sterile water
- Isopropanol
- Ethanol (70 %)

Additional buffers can be purchased separately, catalogue numbers available on request

Troubleshooting

Problem	Possible cause	Corrective action
PCR inhibition	Incomplete buffer removal	Ensure all the buffer is removed before adding the next buffer
Low yield	Poor protease activity	Prepare the protease as detailed in the 'Reagent preparation' section, aliquot into several tubes and store -20 ^{o}C. Remove and thaw aliquots as required. Do not use protease which has been kept at room temperature for an extended period of time
Coloured DNA solution	Inefficient lysis	Ensure that the pellet is fully re-suspended after lysis, if not, vortex thoroughly and prolong lysis for 10 minutes with vortexing before and after lysis until the pellet is fully re-suspended
	Blood clots	Remove any clots from the blood prior to starting

Tips for manual protocol

- The PLUS XL manual kit has been optimised to extract DNA from 2 to 10 mL of blood. The protocol can be scaled down for smaller volumes of blood (and plastics), however care should be taken to maintain the ratio of buffer volumes as specified in the enclosed protocol
- If lysis mixture is not clear prolong the lysis, see troubleshooting section.
- If the DNA pellet appears greenish as apposed to white or pale yellow:
    - Dry the pellet then re-suspend in 1 mL of Lysis buffer BLQ
    - Add 20 µL of Protease and incubate at 55 ^{o}C for 1 hour
    - Allow to cool and precipitate the DNA by adding 1 mL of isopropanol
    - Wash the pellet with 1 mL of 70 % ethanol
    - Follow steps 13 to 16 of the protocol

Storage

Kit components should be used within 12 months of delivery and stored under the recommended conditions. Please refer to the kit box label for the expiry date.

Room temperature	4 ^{o}C	-20 ^{o}C
Lysis buffer BLQ Resuspension buffer BL	5x Enrichment buffer BLL	Protease

Reagent preparation

Enrichment buffer BLL

Prepare the 1.25x Enrichment buffer BLL according to the instructions on the bottle label. For kit catalogue number 40801 add 225 mL of pure water to 75 mL of 5x Enrichment buffer BLL concentrate and mix well. For kit catalogue number 40810 add 375 mL of pure water to 125 mL of 5x Enrichment buffer BLL concentrate. Mix well. When not in use store the 1.25x Enrichment buffer BLL at 4 ^{o}C.

Protease

Prepare the Protease by adding the appropriate amount (see table below) of pure water to the vial of Protease. When not in use store the Protease at -20 ^{o}C.

Kit catalogue number	Volume of pure water
40801	220 µL
40810	1.1 mL

Manual protocol

1. Bring **1.25x Enrichment buffer BLL** and blood samples to room temperature before starting extraction protocol
2. Add 20 mL of **1.25x Enrichment buffer BLL** to 10 mL of blood in a 50 mL centrifuge tube. Close the tube tightly and mix well by inverting the tube
3. Incubate at room temperature for 2 minutes, the solution will become clear
4. Centrifuge at 2 500 g for 5 minutes. Remove the supernatant carefully without disturbing the pellet. Note: The supernatant must be removed directly after centrifugation otherwise the pellet does not remain at the bottom of the tube and could be lost during the supernatant removal
5. Add 7.5 mL of **1.25x Enrichment buffer BLL** to the pellet and using a vortex ensure the pellet is fully re-suspended
6. Centrifuge at 2 500 g for 3 minutes. Remove the supernatant carefully without disturbing the pellet. Note: The supernatant must be removed directly after centrifugation otherwise the pellet does not remain at the bottom of the tube and could be lost during the supernatant removal
7. Re-suspend the pellet in 5 mL of **Lysis buffer BLQ** and add 20 µL of **Protease**. Mix thoroughly using a vortex
8. Incubate at 60 ^{o}C for 10 minutes then allow to cool to room temperature
9. Add 5 mL of **isopropanol** to each sample
10. Mix the tubes carefully by inverting the tube at least 20 times, a white fibrous precipitate should form
11. Centrifuge at 2 500 g for 5 minutes. Remove the supernatant carefully without disturbing the pellet
12. Add 5 mL of **70 % ethanol** to each sample and briefly vortex
13. Centrifuge at 2 500 g for 5 minutes. Remove the supernatant carefully without disturbing the pellet
14. Leave tubes open at room temperature for 10 minutes to let any residual ethanol evaporate
15. Add 1 mL of **Resuspension buffer BL** to each sample and briefly vortex
16. Incubate at 65 ^{o}C for 1 hour. Note: To ensure complete re-suspension of the DNA incubate at 55 ^{o}C overnight.