

## Blood DNA Preparation Kit

### DNA purification from whole blood

#### Solution based genomic DNA Preparation Kit

	<b>Cat.-No.</b>	<b>Amount</b>
	PP-205XS	20 preparations
	PP-205S	100 preparations
	PP-205L	500 preparations

For *in vitro* use only.  
Quality guaranteed for 12 months.  
Store at room temperature.

#### Kit contents

RBC Lysis Solution  
Cell Lysis Solution  
Protein Precipitation Solution  
Washing Buffer (before use, add 96-99% Ethanol as indicated on the bottle)  
DNA Hydration Solution

#### To be provided by you

Isopropanol (2-propanol) >99%  
96-99% Ethanol  
Microtubes 1.5 or 2.0 ml  
Heating Block or Water Bath at 65°C

#### Description

Blood DNA Preparation Kit is designed for convenient and fast isolation of genomic DNA from whole blood samples. The solution based system minimizes DNA fragmentation that may be problematic in spin-column / filtration based methods. Because phenol or chloroform is not used it is safe and does not produce any harmful waste.

Solution based genomic DNA purification kits guarantee minimal DNA fragmentation and yield DNA sized up to 150 kb.

#### Expected yield

Yields of genomic DNA will vary from sample to sample depending on the amount, quality and type of material processed. An amount of approx. 10-20 µg purified DNA can be expected per preparation of 300 µl whole blood. Upscaling of the preparation by a factor of 10 is easily possible if larger amounts of DNA are required.

<b>Buffer</b>	<b>PP-205XS</b>	<b>PP-205S</b>	<b>PP-205L</b>
	20 preps	100 preps	500 preps
RBC Lysis Solution	19.2 ml	96 ml	2 x 240 ml
Cell Lysis Solution	6.4 ml	32 ml	160 ml
Protein Precipitation Solution	2.2 ml	11 ml	55 ml
Washing Buffer	add 9.6 ml Ethanol (final volume 12 ml)	add 48 ml Ethanol (final volume 60 ml)	add 120 ml Ethanol to each bottle (final volume 150 ml each)
DNA Hydration Solution	2.2 ml	11 ml	55 ml

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#### Preparation procedure

Before start, provide >99% Isopropanol (2-propanol) (not included in the kit).

**For XS pack (20 preps):** Add 9.6 ml 96-99% Ethanol (not included in the kit) to the *Washing Buffer* bottle.

**For S pack (100 preps):** Add 48 ml 96-99% Ethanol (not included in the kit) to the *Washing Buffer* bottle.

**For L pack (500 preps):** Add 120 ml 96-99% Ethanol (not included in the kit) to each *Washing Buffer* bottle.

#### 1 Cell Lysis

- Add 300 µl of whole blood (or bone marrow) to a 1.5 ml microtube containing 900 µl *RBC Lysis Solution* and invert 10 times.
- Incubate for 3 min at room temperature with occasional inversion. (Please Note: For fresh blood collected within 1 hour before preparation increase the incubation time to 10 min to ensure complete red blood cell lysis.)
- Centrifuge for 30 sec at 15,000 g.
- Remove the supernatant with a pipet leaving behind the visible white cell pellet and about 10-20 µl of the residual liquid.
- Vortex the tube vigorously for 10 sec to resuspend the white cells in the residual liquid. (The white cell pellet should be completely resuspended.)
- Add 300 µl *Cell Lysis Solution* to the resuspended cells and pipet up and down to lyse the cells until no clumps are visible.
- For heparin-treated blood, heat the white cell pellet for 10 min at 65°C to facilitate lysis.

#### 2 Protein Precipitation

- Add 100 µl *Protein Precipitation Solution* to the cell lysate.
- Vortex vigorously for 20 seconds to mix well.
- Centrifuge at 15,000 g for 1 min.
- The precipitated proteins should form a tight, dark brown pellet. (If the protein pellet is not tight, repeat vortexing, followed by incubation on ice for 5 min and centrifuge again.)

#### 3 DNA Precipitation

- Transfer the supernatant into a clean 1.5 ml microtube containing 300 µl *Isopropanol >99%*.
- Mix the sample by inverting gently for 1 min.
- Centrifuge at 15,000 g for 1 min. (DNA should be visible as a small white pellet.)
- Discard the supernatant and drain tube briefly on clean absorbent paper.
- Add 500 µl *Washing Buffer* and invert the tube several times to wash the DNA pellet.
- Centrifuge at 15,000 g for 1 min.
- Carefully discard the ethanol and dry at room temperature for about 10 to 15 min.

#### 4 DNA Hydration

- Add 50-100 µl *DNA Hydration Solution*.
- Vortex 5 sec at medium speed to mix.
- Incubate the sample at 65°C for 30 min to accelerate rehydration.
- Store DNA at 4°C. For long time storage, place sample at -20°C or -80°C.