Dvysr®

Handbook

Devyser Genomic Blood Typing

Art. no. 8-A419-24 | 8-A419-96

For research use only

7-A158-en-US-2025-07-01, RUO

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1 Introduction

Handbook update service

Sign up for the handbook update service to receive notifications via e-mail whenever there is a new version of the handbook available.

Visit *https://www.devyser.com/ifu-subscription* to sign up.

1.1 Description

Devyser Genomic Blood Typing is a qualitative next-generation sequencing assay designed to determine blood cell antigens from human genomic DNA (gDNA).

NOTE

For research use only. Not for use in diagnostic procedures.

1.2 Background

Blood typing refers to analyzing the presence or absence of surface antigens on blood cells to determine an individual's blood type. Defining blood cell antigens is crucial in transfusion research, as it aims to investigate immune responses to mismatched cells and compatibility mechanisms. Currently, the standard approach relies on serological methods to type an individual's blood cell antigens. While serological blood typing is effective in identifying the most common red blood cell antigens (blood groups), such as those found in the ABO and Rh (Rhesus) systems, these methods may not provide complete results for all blood group systems (1). Consequently, complications arising from the mismatching of the less prominent surface antigens on red blood cells remain an issue (2,3). Similarly, blood cell antigens on the surface of platelets and neutrophils may present molecules with immunogenic effect, such as HPA (human platelet antigens) and HNA (human neutrophil antigens) (4,5), respectively.

Analysis of the genetic alterations underlying different blood cell antigens supports more precise prediction of a person's blood cell profile and can facilitate insights beyond serology. Molecular blood typing can support comprehensive and rapid blood cell antigen characterization and resolve complex scenarios (6).

1.3 Assay principle

The Devyser Genomic Blood Typing assay is an amplicon-based target enrichment panel for NGS that employs gDNA as input material.

The method employed by the Devyser Genomic Blood Typing assay includes multiplex PCR amplification of human gDNA (PCR1) to create a target amplicon library of genomic regions from each DNA sample as schematically illustrated in Figure 1.

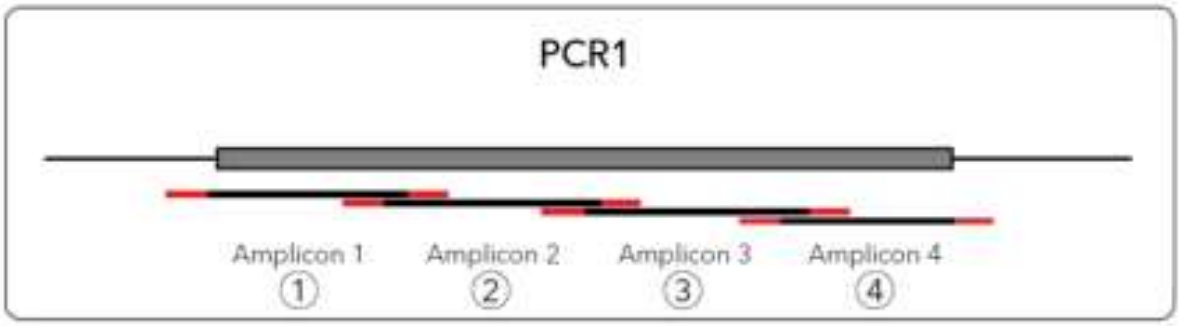


Figure 1. Schematic illustration of PCR1

1. Target 1 – Amplicon
2. Target 2 – Amplicon
3. Target 3 – Amplicon
4. Target 4 – Amplicon

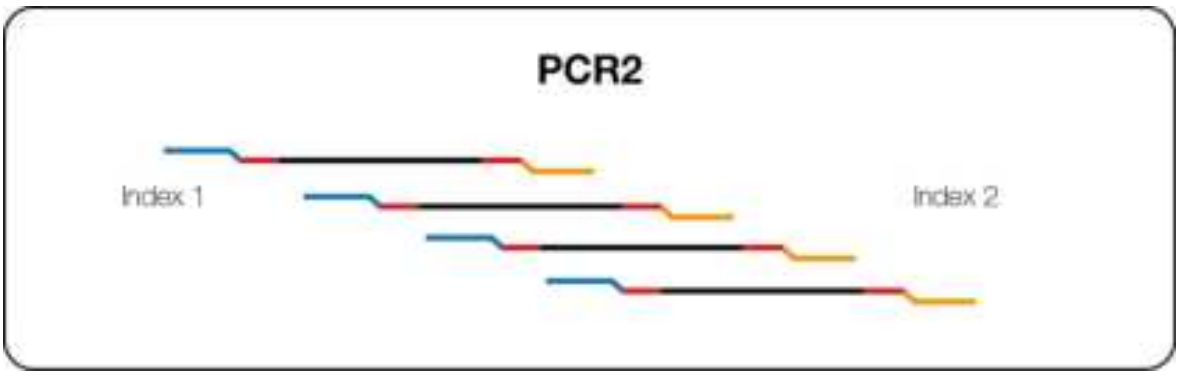


Figure 2. Schematic illustration of PCR2 Assay design

In a second PCR reaction (PCR2), sequencing adapters including unique index sequences are introduced into each amplicon (Figure 2), enabling pooling of several samples. The purified sample pool is sequenced using NGS chemistry and the resulting sequences are analyzed using Advyser Blood Suite as described in chapter 9.

The Devyser Genomic Blood Typing assay enables library generation for NGS analysis of:

- 28 genes encoding 22 red blood cell (RBC) antigen systems or blood group systems.

Table 1. Genes and blood group systems detected with Devyser Genomic Blood Typing

Gene	Blood group system	Covered regions
ABO	ABO	All 7 exons + intron 1
AQP1	Colton	Exons 1 (part), 3
CD55	Cromer	Exons 2-6 (2-3 partly)
SLC4A1	Diego	Exons 14, 16, 19
ART4	Dombrock	Exons 1-2
ACKR1	Duffy	All 2 exons
GYPC	Gerbich	All 4 exons
FUT1	H (ABO precursor)	Part of exon 2
FUT2	H (ABO precursor)	Part of exon 1
SLC35C1	H (ABO precursor)	Part of exons 1 and 2
CD44	Indian	Exons 2 (part) and 3
SEMA7A	JohnMiltonHagen	Exons 6, 9, 11
KEL	Kell	All 19 exons
SLC14A1	Kidd	All 8 coding exons
CR1	Knops	Part of exon 29
BCAM	Lutheran	Exons 2, 3, 5-7, 10-13
ICAM4	Landsteiner-Wiener	Part of exon 1
GYPA	MNS	Exons 1-6
GYPB	MNS	All 5 exons + 1 pseudoexon
BSG	Ok	Exon 4
A4GALT	P1PK	Regulatory intron 2
RHD	Rh	All 10 exons
RHCE	Rh	All 10 exons
RHAG	Rh-associated glycoprotein	All 10 exons
ERMAP	Scianna	Part of exon 4
SMIM1	Vel	Part of intron 2 and Exons 3, 4
ACHE	Yt	2 parts of exon 2

- Relevant regions of 6 genes (32 antigens) for Human Platelet Antigen (HPA) systems: HPA-1 through HPA-28, HPA-32 through HPA-35.

Table 2. Genes and HPA systems detected with Devyser Genomic Blood Typing

Gene	Human platelet antigen systems	Covered regions
ITGB3	HPA-1, -4, -6, -7, -8, -10, -11, -14, -16, -17, -19, -21, -23, -26, -32, -33, -34, -35	Exons 3-5, 10-12
GP1BA	HPA-2	Part of exon 1
ITGA2B	HPA-3, -9, -20, -22, -24, -27, -28	Exons 5, 6, 15, 20, 23, 26
ITGA2	HPA-5, -13, -18, -25	Exons 13, 17, 20, 28
GP1BB	HPA-12	Part of exon 2
CD109	HPA-15	Exon 19

- Relevant regions of 5 genes (5 antigens) for Human Neutrophil Antigens (HNA) systems: HNA-1 through HNA-5.

Table 3. Genes and HPA systems detected with Devyser Genomic Blood Typing

Gene	Human neutrophil antigen systems	Covered regions
FCGR3B	HNA-1	Exon 3
CD177	HNA-2	Exon 7 (wildtype and pseudoexon)
SLC44A2	HNA-3	Exon 7
ITGAM	HNA-4	Exon 3
ITGAL	HNA-5	Exon 21

2 Materials and equipment

2.1 Kit configuration

Note.

The individual components of the kit are not available for purchase separately.

- 24 tests: 8-A419-24
- 96 tests: 8-A419-96

Table 4. Devyser Genomic Blood Typing, 24-test (8-A419-24)

Component	Art. No.	Number/kit	Cap color	Storage condition
GBT Mix	4-A375	1	Blue	Below –18°C
Start GBT	4-A376	1	Purple	Below –18°C
Dilution buffer	4-A245	3	White	–25°C to +8°C
Index mix S	4-A302	1	Red	Below –18°C
Index buffer	4-A258	3	Green	–25°C to +8°C

Table 5. Devyser Genomic Blood Typing, 96-test (8-A419-96)

Component	Art. No.	Number/kit	Cap color	Storage condition
GBT-mix	4-A375	4	Blue	Below –18°C
Start GBT	4-A376	4	Purple	Below –18°C
Dilution buffer, 96-test	4-A275	1	White	–25°C to +8°C
Index mix S	4-A302	4	Red	Below –18°C
Index buffer, 96-test	4-A277	1	White	–25°C to +8°C

2.2 Equipment and reagents required but not provided

2.2.1 Other required Devyser products

- Devyser Library Clean (8-A204)
- Devyser Index plate A (8-A200)

Table 6. Devyser Library Clean (8-A204)

Component	Art. No.	Number/kit	Cap color	Storage condition
Clean	4-A255	1	Orange	+2°C to +8°C
Wash	4-A256	1	Yellow	+2°C to +8°C
Dilution buffer	4-A245	1	White	+2°C to +8°C

Table 7. Devyser Index plate A (8-A200)

Component	Art. No.	Number/kit	Cap color	Storage condition
Index Plate A3	4-A253	1	-	Below -18 °C
Sealer L	6-A040	2	-	Ambient

2.2.2 General

- Micropipette, multipipette, or dispenser with aerosol barrier tips for pre-PCR and post-PCR
- Disposable powder-free protective gloves
- Reaction tubes

2.2.3 Determination of DNA concentration

- Qubit 1X dsDNA HS Assay Kit (Thermo Fisher Scientific) or equivalent fluorometric measurement technique enabling robust quantification below 1ng/µl.
- Consumables according to manufacturer's instructions for use

2.2.4 Target amplification

- Veriti Thermal Cycler with MicroAmp 96-Well Tray/Retainer Set (Thermo Fisher Scientific)
- If an alternative thermal cycler is used, a thorough evaluation of its performance should be performed. It is of high importance that the following ramp rates are applied: heating 1,6 ˚C/s, cooling 1,6 ˚C/s
- Consumables for the thermal cycler

2.2.5 Library purification

- Magnetic rack for test tubes (DynaMag-2 Magnet, Thermo Fisher Scientific or equivalent)
- Ethanol (96%)

2.2.6 Sequencing

- Illumina MiSeq
- Other user-supplied consumables needed for sequencing, according to Illumina's sequencing guide
- Illumina reagent kits (Table 8)

Table 8. Illumina MiSeq reagent kits

Illumina reagent kit
MiSeq Reagent Kit v2 (300 cycles)
MiSeq Reagent Micro Kit v2 (300-cycles)
MiSeq Reagent Nano Kit v2 (300-cycles)
Illumina PhiX control v3

NOTE

All equipment should be tested, calibrated, and maintained regularly.

2.3 Software

The sequencing data is analyzed using the supported software Advyser Blood Suite. The software features quality control information and multi-stage analysis: from antigen insights to in-depth variant exploration, such as indels, SNVs, and CNVs.

2.4 Downloads

Download additional information and files from www.devyser.com/ifu using the access code or batch/lot number provided on the kit label. See Table 9 for details.

See also Download update service, section 1, page 4.

Table 9. Supplementary information

Download file name	Description
Devyser Genomic Blood Typing RUO 7-A158	Handbook
BRC	Batch Release Certificate
LRM files	Devyser setting files for MiSeq sample sheet generation using LRM: • Generating a Devyser sample sheet with Illumina LRM.doc • Devyser.tsv files
MiSeq IEM files	Devyser setting files for MiSeq sample sheet generation using IEM: • Generating a Devyser sample sheet for MiSeq®.doc • DEVYSER double Index MiSeq®.txt • DevyserGenerateFASTQ.txt • DevyserGenerateFASTQ.jpg
Devyser Genomic Blood Typing BED files	BED files detailing amplicon positions according to Hg38/GRCh38
Advyser Blood Suite RUO	Software handbook

2.5 Other resources

2.5.1 Devyser sequence coverage calculator

To plan the sequencing run with respect to coverage needs, please consult the Devyser sequence coverage calculator at www.devyser.com/calculator.

As displayed in the Devyser sequence coverage calculator, the recommended number of single reads per sample and coverage per amplicon is according to the table below.

Table 10. Minimal recommended number of single reads per sample and amplicon coverage

Detection mode	Read pairs per sample	Coverage per amplicon
Germline	91 000	200

- After the sequencing run, the sequencing read and coverage information can be found in Illumina Sequencing Analysis Viewer (SAV), BaseSpace or Local Run Manager (LRM), as well as in the Advyser Blood Typing software.

3 Storage requirements

- Store the Devyser Genomic Blood Typing kit below −18°C (−28°C to −18°C) or the individual kit components as specified on the label, see 2.1 Kit configuration, page 8
- Store the components of the Devyser Library Clean kit at +2°C to +8°C
- Do not store the kit in a self-defrosting freezer
- Do not use components beyond the kit expiration date
- If handled, reclosed and stored properly, kit components will remain stable until the expiration date of the kit or according to in-use stability
- Frozen kit components should be thawed in a refrigerator or at room temperature before use
- Avoid repeated freeze-thaw cycles of all kit components

4 Warnings and precautions

- Use of this product should be limited to personnel trained in PCR, NGS techniques and NGS data analysis
- The procedure should be performed according to this guide
- Wear powder-free disposable gloves, laboratory coat and eye protection when handling samples and kit reagents
- Do not pool reagents with different batch numbers or different vials of the same batch
- Do not use reagent vials from a new kit if they are damaged or have already
- been opened
- Frozen components should be completely thawed in a refrigerator or at room temperature before use
- Use, storage and disposal of kit components and samples, must be in accordance with the procedures defined by national biohazard safety guidelines and in accordance with country, federal, state and local regulations
- Avoid microbial contamination of reagents when removing aliquots from reagent vials
- The use of sterile disposable aerosol barrier pipette tips is recommended
- The use of different sets of pipettes for the initial addition of DNA samples and for diluting and handling samples after PCR amplification is recommended.
- Highly concentrated amplicons produced during PCR amplification must be handled with care to avoid contamination in the laboratory environment
- The workflow in the laboratory should proceed in a unidirectional manner, beginning in the reagent preparation area, moving to the DNA extraction area, then to the amplification area and finally to the sequencing area
- Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas
- Use different indexes in consecutive runs to avoid carry-over contamination in Illumina sequencing instruments
- Change gloves between activities

5 Procedural limitations

- This product is intended for research use only. Not for use in diagnostic procedures
- The following parameters might affect the overall performance:
    - Quality and concentration of the DNA
    - Deviations from the protocol
    - Too low sequencing coverage
- The quality of CNV results can be hampered by low quality DNA and/or non-optimal PCR reactions like evaporation.
- Rare primer site sequence alterations may affect the function of individual PCR primers used in the Devyser Genomic Blood Typing kit and may result in reduced or no amplification of the affected amplicon
- Homology between different genes e.g. *RHD*/*RHCE* or *GYPA*/*GYPB*/*GYPE* can cause wrong allele identification if variants and/or hybrid genes lead to alignment of exons/amplicons to the "wrong" gene.
- Devyser Genomic Blood Typing may not detect all rare or novel alleles
- Devyser Genomic Blood Typing may show limitations in performance when samples are analyzed from a recipient who has had a blood transfusion that contains white blood cells within the past 30 days
- Do not use the Devyser Genomic Blood Typing kit to analyze samples from a transplant recipient who has had HSCT procedure(s) and mixed chimerism can be suspected
- Phasing challenges in the *ABO* and *RHD*/*RHCE* genes, and hybrid variants in the *RHD*/*RHCE* genes, may lead to incorrect allele calls
- Devyser Genomic Blood Typing is meant to complement traditional serological methods and should not be used as a standalone test

6 Sample requirements

DNA concentration, integrity and purity are important parameters for successful testing using Devyser Genomic Blood Typing. DNA should be free from contaminating proteins, salts and other PCR inhibitors, e.g., residual ethanol from DNA extraction procedures. Poor quality DNA may result in amplification failure and/or increased background signals.

6.1 Samples

The Devyser Genomic Blood Typing kit has been evaluated using human genomic DNA extracted from whole blood.

6.2 DNA extraction

According to manufacturer's instructions for use.

6.3 Determination of DNA concentration

- High quality DNA is important for accurate and reproducible determination of DNA concentration
- All DNA concentrations referred to in this handbook were determined using the Qubit Fluorometer and the Qubit 1X dsDNA HS Assay Kit
- The DNA concentration determined for a DNA sample may differ between Qubit systems and between the Qubit system and other techniques. It is important to verify that the technique used for determination of DNA concentration correlates to the actual results obtained with the Devyser Genomic Blood Typing kit

6.4 Dilution of DNA

Adjust the concentration of extracted DNA to 2 ng/µL using the Dilution buffer provided with the kit (see 7.1.2, page 18)

6.5 PhiX control

Include PhiX control (section 2.2.6, table 8, page 9) in each sequencing run to create quality parameters for the sequencing run.

7 Instructions for use

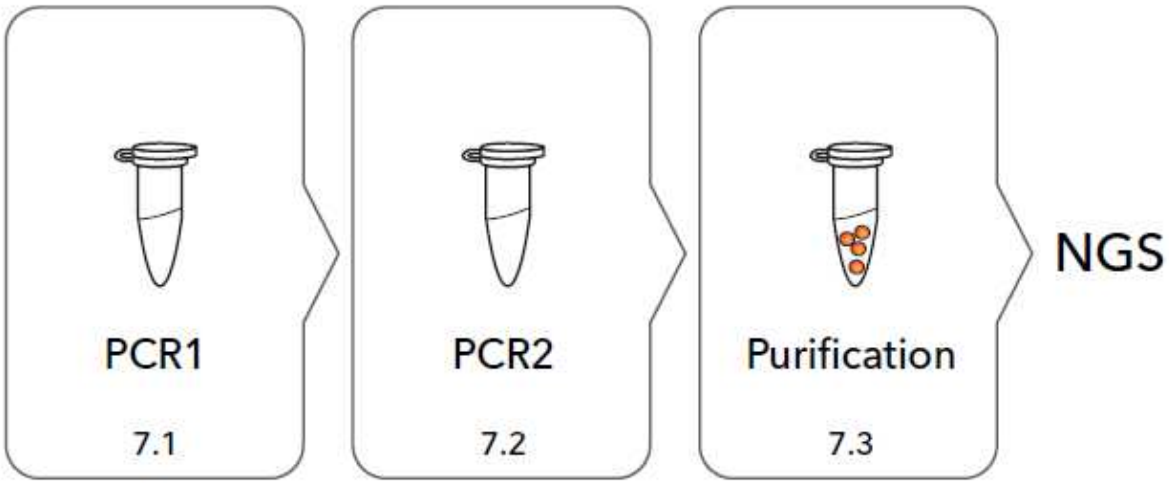


Figure 3. Schematic overview of the Devyser Genomic Blood Typing library preparation procedure (sections 7.1 to 7.3.6)

The Devyser Genomic Blood Typing library preparation procedure consists of the following steps:

PCR1

The amplicon library is generated in one multiplex PCR reaction for each sample.

PCR2

Index addition to the PCR1 library is performed in PCR2 to allow pooling of multiple sample libraries for sequencing.

Purification

Unique sample libraries generated in PCR2 are pooled and purified in a single tube. The purified library pool is analyzed by NGS.

Each step (7.1 to 7.3) is followed by a suitable stopping point where the procedure can be paused and restarted within 30 days.

7.1 Library generation (PCR1)

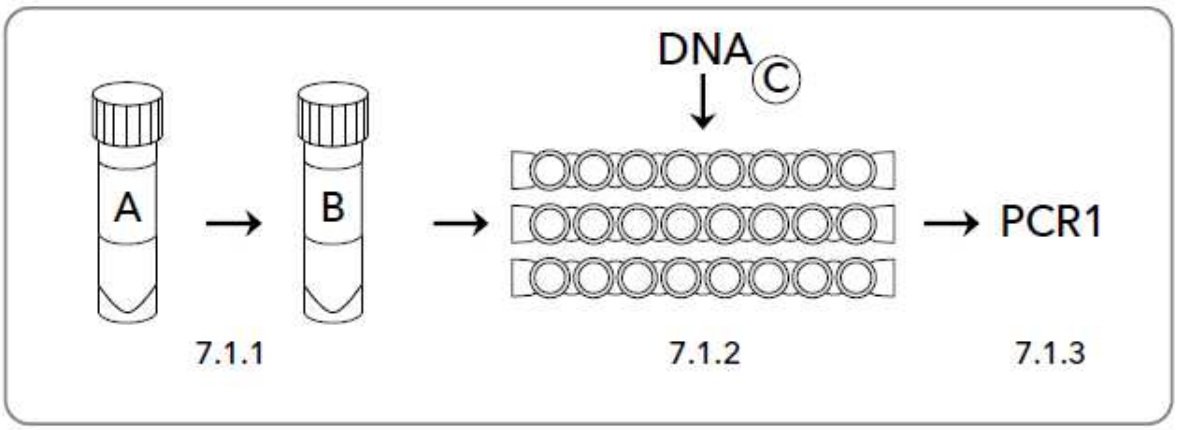


Figure 4. Schematic overview of 7.1.1 to 7.1.3

A. Start GBT
B. GBT mix
C. DNA

7.1.1 Activation of GBT mix

Required kit components:

24/96 kit: **Start GBT, GBT mix**

Determine the number of **Start GBT** and GBT mix tubes required. Each tube is sufficient for 24 reactions.

A. Ensure that the **Start GBT** and the **GBT mix** are completely thawed before use
B. Vortex the **Start GBT** tube(s) briefly
C. Briefly centrifuge the **Start GBT** and **GBT mix** tube(s) to collect the content
D. Add 150 µL of **Start GBT** to the **GBT mix** tube(s) to obtain an activated **GBT mix**
E. Vortex the activated **GBT mix** tube(s) and then centrifuge it briefly to collect the content
F. Dispense 10 µL of the activated **GBT mix** into separate PCR reaction tubes or separate wells in a PCR plate. Cap the tubes or seal the plate
G. Store the dispensed **GBT mix** at +2 °C to +8 °C and continue to 7.1.2
H. Any remaining activated **GBT mix** can be stored in a freezer below −18 °C for 60 days. Do not aliquot the activated mix

7.1.2 Preparation and addition of DNA

Required kit components: **Dilution buffer** (4-A245)

A. Determine the DNA concentration of each DNA sample (see 6.3, page 15)
B. Ensure that the **Dilution buffer** is completely thawed before use
C. Dilute the DNA samples to a final concentration of 2 ng/µL using the provided Dilution buffer
D. Add 5 µL of diluted DNA from each sample to the separate PCR reaction tubes or the separate wells in the PCR plate containing the activated **GBT mix** (from 7.1.1)
E. Mix by pipetting
F. Cap the tubes or seal the plate and centrifuge briefly to collect the content

7.1.3 Amplification PCR1

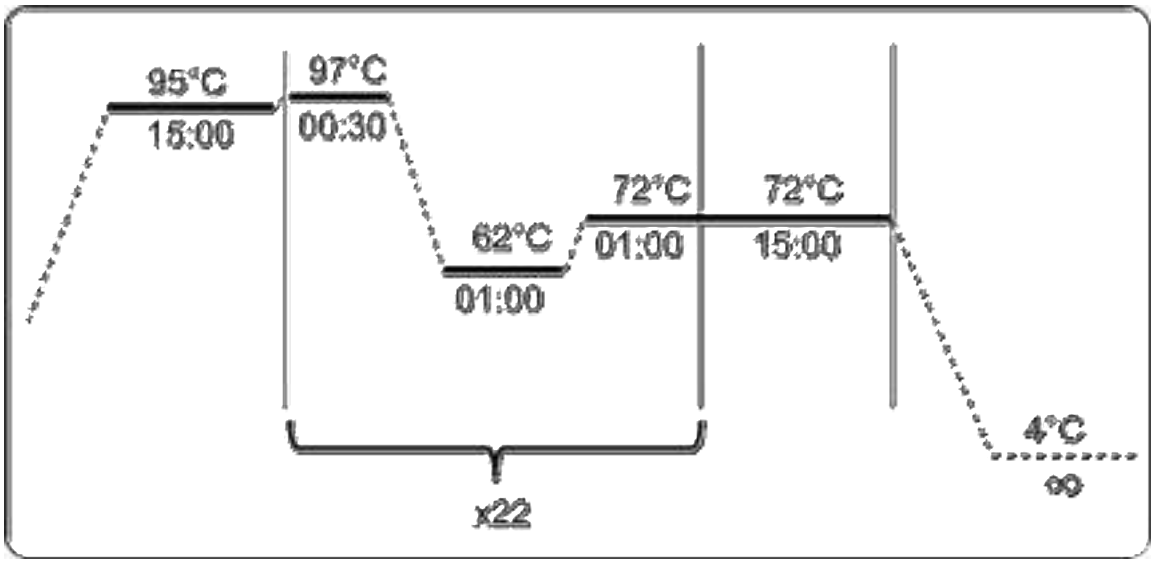


Figure 5. Thermal profile PCR1

A. Program the Veriti Thermal Cycler:
   - Create a new method with the following sample ramp rate: **heating 1.6°C/s, cooling 1.6°C/s**

     Veriti Thermal Cycler:

     a. In the "Tools Menu" select "Convert a Method"

     b. Select "9700 Max Mode"

     VeritiPro Thermal Cycler:

     a. Create a new method following the sequence:
     New method → Open template → Blank Template → General PCR

b. Set the sample ramp rate following the sequence:
Actions → Simulation mode → GeneAmp PCR System 9700

B. Set the reaction volume to 15 µL
C. If using tubes/strips in a Veriti or Verity Pro Thermal Cycler they should first be placed in the MicroAmp 96-Well Tray/Retainer Set for Veriti Systems
D. Place the tubes or the plate in the thermal cycler
E. Start the amplification (duration approximately 1 hr 45 min)
F. Following amplification, centrifuge briefly to collect the content.
G. The PCR1 library can be stored in a freezer below −18°C for 30 days.

NOTE

Do not manually program sample ramp rates in the PCR profile.

NOTE

Suitable stopping point.

7.2 Library indexing (PCR2)

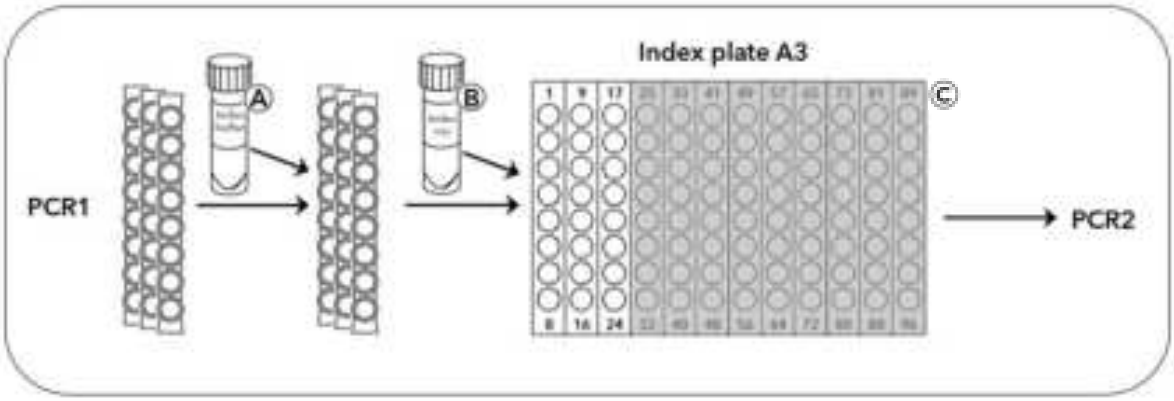


Figure 6. Schematic overview representing 24-reaction kit utilizing 3 columns of the Index plate A3 7.2.1 to 7.2.4

A. Index buffer

B. Index mix

C. Devyser Index Plate A

7.2.1 PCR1 library dilution

Required kit components: **Index buffer** (4-A258)

A. Ensure that the **Index buffer** is completely thawed before use
B. For each PCR1 library to be diluted, dispense 198 µL **Index buffer** to a new tube
C. Add 2 µL of each PCR1 library to the separate dilution tubes containing 198 µL **Index buffer**. Ensure no liquid remains in the tip by pipetting repeatedly in the **Index buffer**
D. Mix the diluted PCR1 libraries thoroughly by pipetting (using a pipetting volume of at least 100 µL)

7.2.2 Index preparation

NOTE

To avoid potential carry over, it is highly recommended to select indexes that have not been seen used in the previous sequencing run.

Required kit component: **Index mix** (4-A247)

Required kit: **Devyser Index Plate A (8-A-200)**

A. Determine the number of **Index mix** tubes required. Each tube is sufficient for 24 reactions
B. Determine the number of indexes required and cut the desired wells from the **Devyser Index Plate**. Store the unused wells below −18°C for a maximum of 24 months or until the expiration date
C. Ensure that the **Index mix** is completely thawed before use
D. Vortex and then briefly centrifuge the **Index mix** tube to collect the content
E. Carefully remove the transport seal from the wells selected in *Step A*. **Do not reuse the transport seal**
F. Add 20 µL of **Index mix** to each well. **Tips must be changed between each individual well**

7.2.3 Addition of diluted PCR1 libraries to index plate

Required kit component: **Sealer L**

A. Add 5 µL of each diluted PCR1 library (from 7.2.1) to separate wells in **Devyser Index plate A**

(prepared in 7.2.2)

B. Mix thoroughly by pipetting to dissolve the colored reagent pellets, using a pipetting volume of at least 10 µL
Make sure that the colored reagent pellets are completely dissolved before proceeding to the next step. Avoid bubbles

C. Cut a piece of **Sealer L** to completely cover the **Devyser Index Plate**, or cut a piece of Sealer L to cover wells in use

D. Carefully seal **Devyser Index Plate** and make sure that all wells are covered

E. Centrifuge briefly to collect the content

7.2.4 Amplification PCR2

A. Program the Veriti Thermal Cycler using the approach described in section 7.1.3
Make sure that the following ramp rates are applied: heating 1,6 ˚C/s and cooling 1,6 ˚C/s

> **NOTE**
>
> Do not manually program sample ramp rates in the PCR profile.

B. Enter the PCR profile outlined in the figure below

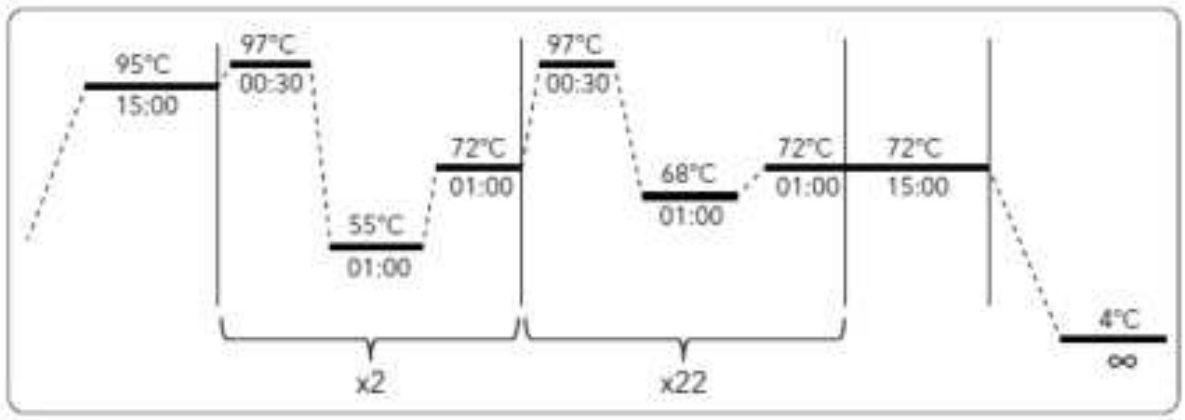


Figure 7. Thermal profile PCR2

C. Set the reaction volume to 25 µL

D. If using tubes/strips in a Veriti or VeritiPro Thermal Cycler, they should first be placed in the MicroAmp 96-Well Tray/Retainer Set for Veriti Systems

E. Place the tubes or the plate in the thermal cycler

F. Cover the tubes or plate with a compression pad (see Figure 8) and close the lid of the thermal cycler

G. Start the amplification (duration approximately 1 h 55 min)

H. If proceeding with sequencing the same day, prepare sequencing reagents (see note in 7.3, page 23)
I. Following amplification, centrifuge briefly to collect the content
J. PCR2 libraries can be stored in a freezer below −18°C for 30 days.

NOTE

Suitable stopping point.

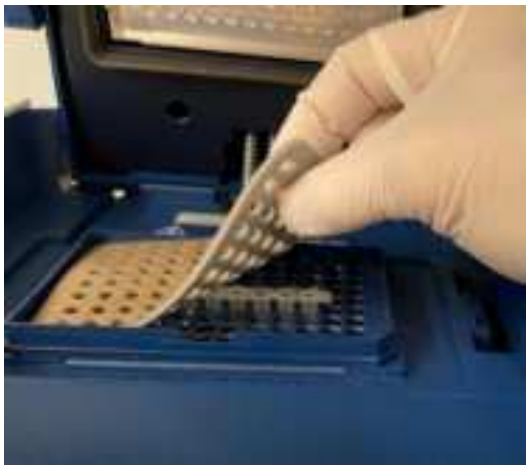


Figure 8. Applying the compression pad before PCR2

7.3 Pooling and purification of libraries using Devyser Library Clean kit

> **NOTE**
>
> Defrost the Illumina reagent cartridge well in advance of sequencing according to the procedure described in the current version of the MiSeq System Guide (7).

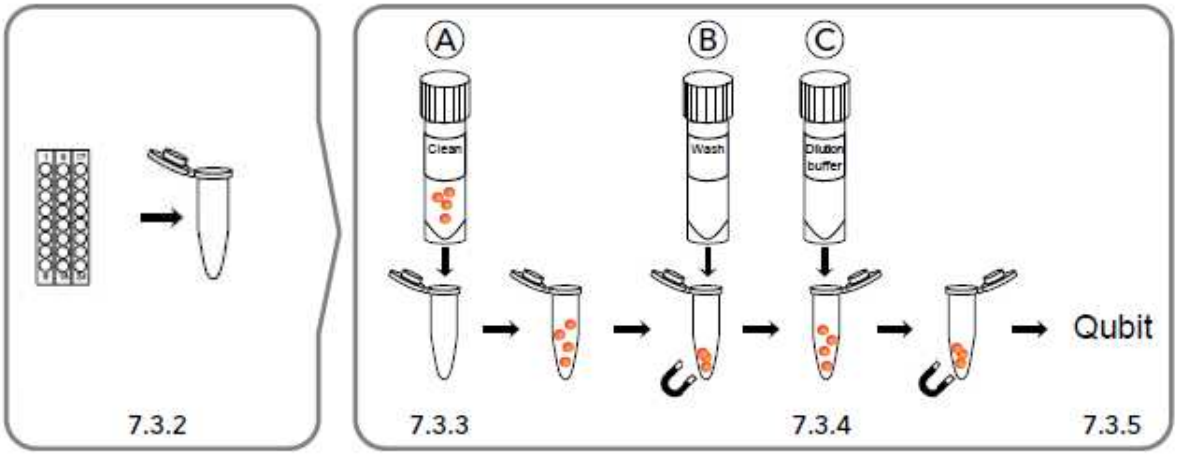


Figure 9. Schematic overview of *7.3.2* to *7.3.5*

A. Clean

B. Wash

C. Dilution buffer

7.3.1 Preparation of wash solution

Required kit: **Devyser Library Clean** (8-A204)

Required kit component: **Wash** (4-A256)

A. Prepare the **Wash** solution by adding 1500 µL of 96% ethanol to the **Wash** tube
B. Mix thoroughly by vortexing
C. Tick the box on the **Wash** tube label to indicate that ethanol was added

The Wash solution should be stored at +2°C to +8°C and used within 3 months from day of preparation

7.3.2 Library pooling

> **NOTE**
>
> The library pool should consist of libraries from samples processed together with the same Devyser library kit.

A. Pool equal volumes, using a pipetting volume of at least 5 µL, from each of the PCR2 libraries (from 7.2.4) into a single tube to obtain a library pool for subsequent purification. Ensure that the volume of the library pool is at least 45 µL
B. Mix thoroughly by vortexing and then briefly centrifuge the library pool to collect the content
C. Transfer 80 µL of the library pool into a new tube suitable for placing on a magnetic rack

> **NOTE**
>
> If less than 16 libraries are pooled, add equal volumes of each PCR2 library to obtain a library pool volume of at least 80 µL.
>
> If the total pooled volume is less than 80 µL, use equal volumes of the pooled PCR2 libraries and Clean.
>
> For elution, use half the library pool volume of Dilution buffer (see Library elution, page 25). However, do not use less than 25 µL of Dilution buffer for elution.

7.3.3 Library purification

Required kit: **Devyser Library Clean** (8-A204)

Required components: **Clean** (4-A255), **Wash** (4-A256)

A. Briefly centrifuge the **Clean** tube to collect the content
B. Firmly flick the **Clean** tube. Make sure that the bead pellet is re-suspended and that the content is homogenous. If necessary, briefly vortex the tube but avoid extensive vortexing
C. Add 80 µL re-suspended **Clean** to the library pool from 7.3.2 and mix by pipetting. See note for use of alternative volumes of library pool and **Clean**
D. Incubate the tube at room temperature for 3 minutes
E. Place the tube onto a magnetic rack until all beads are pelleted and the solution is clear
F. While keeping the tube on the magnetic rack, carefully remove and discard the supernatant.
It is important to avoid touching the bead pellet during this step (see Figure

10)

G. Add 150 µL of prepared **Wash** solution (from 7.3.1) to the tube without removing it from the magnetic rack
H. Slightly lift and rotate the tube two half circles to wash the beads
I. Place the tube onto the magnetic rack to pellet the beads
J. Carefully remove as much **Wash** solution as possible by pipetting from the bottom of the tube
It is important to avoid touching the bead pellet and the walls of the tube during this step
K. Leave the lid open until all remaining **Wash** solution has evaporated and the bead pellet has changed from being luster to lusterless, approximately 3-5 minutes, while remaining on the magnetic rack. **Important! See note below**
L. Remove the tube from the magnetic rack

> **NOTE**
>
> It is important that all Wash solution has evaporated before continuing.
>
> If Wash solution remains, briefly centrifuge the tube to collect all remaining Wash solution, pellet the beads using the magnetic rack, remove the residual Wash solution and air dry the pellet again.

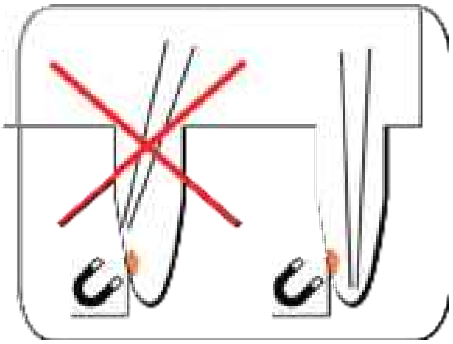


Figure 10. Bead pellet

7.3.4 Library elution

Required kit: **Devyser Library Clean** (8-A204)

Required kit component: **Dilution buffer** (4-A245)

A. Briefly centrifuge the **Dilution Buffer** tube to collect the content
B. Add 40 µL **Dilution buffer** to the tube prepared in 7.3.3 and re-suspend the pellet by pipetting and/or tapping the tube. If necessary, collect the liquid by a brief centrifugation
C. Place the tube(s) onto the magnetic rack until all beads are pelleted

D. While keeping the tube(s) on the magnetic rack, transfer the cleared supernatant, containing the purified library pool(s), to a new tube

7.3.5 Library quantification

Required kit: **Qubit 1X dsDNA HS Assay Kit** (see Determination of DNA concentration, section 2.2.3, page 9)

Quantify the library as described in the valid user manual for Qubit 1X dsDNA HS Assay Kits.

A. Ensure that all Qubit 1X dsDNA HS solutions are at room temperature
B. Mix 190 µL Qubit 1X dsDNA HS working solution with 10 µL of Qubit standard 1
C. Mix 190 µL Qubit 1X dsDNA HS working solution with 10 µL of Qubit standard 2
D. Mix 190 µL Qubit 1X dsDNA HS working solution with 10 µL of the purified library pool from 7.3.4
E. Briefly vortex, centrifuge and incubate each tube for 2 minutes at room temperature
F. Measure the concentration (ng/µL) of the purified library pool on a Qubit Fluorometer

7.3.6 Library dilution

Required kit: **Devyser Library Clean** (8-A204)

Required kit component: **Dilution buffer** (4-A245)

A. Dilute the purified library pool from 7.3.4 to a final concentration of 0.38-0.42 ng/µL using the **Dilution buffer**
B. Measure the concentration (ng/µL) of the diluted library pool to confirm the concentration by repeating steps E and F in 7.3.5
C. Proceed to sequencing according to section 8

NOTE

The purified and diluted library pool is not recommended to be stored for long term or to be re-used. For potential re-runs it is instead recommended to repeat the pooling and purification step of this protocol from point 7.3.2, to ensure that the library holds the final concentration range defined in 7.3.6.

8 Sequencing

Devyser Genomic Blood Typing can be used with the Illumina MiSeq system. The Devyser Sequence Coverage Calculator can be used to calculate the number of samples to be sequenced per flow cell.

NOTE

Defrost the MiSeq cartridge well in advance prior to sequencing according to the procedure described in the current version of the MiSeq System Guide (7).

8.1 Sequencing mode

The Devyser Genomic Blood Typing libraries should be sequenced in single-read (SR) mode:

- SR: 1 x 301 bp

8.2 Number of samples per flow cell

Calculate the number of samples to be sequenced per flow cell by using the Devyser Sequence Coverage Calculator (see Devyser sequence coverage calculator, section 2.5.1 page 11).

8.3 Sample sheet generation

Generate a sample sheet for each run by using one of the following software:

Illumina Experiment Manager (IEM) software

Use the Devyser "Generating a Devyser sample sheet for MiSeq" guide (see Downloads, 2.4, page 10) and the Illumina Experiment Manager User Guide (8).

Local Run Manager (LRM) software

Use the Devyser "Generating a Devyser sample sheet with Illumina LRM" guide (see Downloads, **section 2.4, page 10**), and the Illumina Local Run Manager v2 Guide (9).

8.4 Index layout

The Illumina double indexes introduced during PCR2 are listed in Table 11 below (see also Downloads, section 2.4, page 10)

Table 11. Illumina indexes used in Devyser Index Plate A.

	Index 1-8	Index 9-16	Index 17-24	Index 25-32	Index 33-40	Index 41-48	Index 49-56	Index 57-64	Index 65-72	Index 73-80	Index 81-88	Index 89-96
	Index1: N701	Index1: N702	Index1: N703	Index1: N704	Index1: N705	Index1: N706	Index1: N707	Index1: N708	Index1: N709	Index1: N710	Index1: N711	Index1: N712
Index2: N501	1	9	17	25	33	41	49	57	65	73	81	89
Index2: N502	2	10	18	26	34	42	50	58	66	74	82	90
Index2: N503	3	11	19	27	35	43	51	59	67	75	83	91
Index2: N504	4	12	20	28	36	44	52	60	68	76	84	92
Index2: N505	5	13	21	29	37	45	53	61	69	77	85	93
Index2: N506	6	14	22	30	38	46	54	62	70	78	86	94
Index2: N507	7	15	23	31	39	47	55	63	71	79	87	95
Index2: N508	8	16	24	32	40	48	56	64	72	80	88	96

NOTE

FASTQ files for MiSeq and MiniSeq index reads are recommended for data analysis.

8.5 Sequencing using MiSeq

8.5.1 Denaturation of the purified library pool

- Prepare 20 pM PhiX, HT1 and a fresh dilution of 0.2 N NaOH according to the current version of the "Denature and Dilute Libraries Guide" (10).
- Combine 5 µL diluted purified library pool from 7.3.6 with 5 µL 0.2 N NaOH
- Briefly vortex, centrifuge and incubate for 5 minutes at room temperature
- Add 1410 µL prechilled HT1 to dilute the denatured library pool
- To obtain a sequencing mix, add 9 µL 20 pM denatured PhiX control DNA to the mixture from above. The added PhiX will represent approximately 1% of the total number of reads from the sequencing run
- Repeatedly invert and then vortex the tube to mix and briefly centrifuge to collect the content

8.5.2 Sequencing

NOTE

All equipment should be tested, calibrated, and maintained regularly.

A. Prepare the sequencing run according to the current version of the "MiSeq System Guide"

B. Add 600 µL of the sequencing mix (8.5.1) to the sample well in the reagent cartridge

C. Load the flow cell and execute the sequencing run using 1 x 301 bp sequencing mode

D. After completion of the sequencing run, locate the generated sequencing data files (FASTQ) and move them to the desired location for analysis

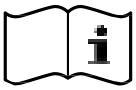
9 Sequence Data Analysis

9.1 Sequence data analysis using Advyser Blood Suite

Data analysis is performed using the Genomic Blood Typing module in Advyser Blood Suite.

Upload the sequencing data files (FASTQ) and start the analysis in Advyser Blood Suite according to manufacturer's instructions for use.

10 Symbols on labels



Consult instructions for use

Lot or batch number

Expiration date

Number of tests

Store below temperature shown

Catalog number

Manufacturer

For Research Use Only. Not for use in diagnostic procedures

11 Notice to purchaser

Purchase of this product does not provide a license to perform PCR under patents owned by any third party.

Devyser® and Dvysr® are registered trademarks of Devyser Diagnostics AB. All trademarks are the property of their respective owner.

12 References

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7. MiSeq System Guide (Document # 15027617).
8. Illumina Experiment Manager Software Guide (Document # 15031335).
9. Local Run Manager Software Guide (Document # 1000000002702).
10. MiSeq System Denature and Dilute Libraries Guide (Document # 15039740).

13 Contact information

13.1 Legal manufacturer

Devyser AB Bränningevägen 12

120 54 Årsta SWEDEN

Phone: +46 8 562 15 850

Homepage: *www.devyser.com*

13.2 Technical support

Phone: +46 8 562 15 850

E-mail: *techsupport@devyser.com*

14 Abbreviations

Abbreviation	Explanation
bp	base pair
CNV	copy number variation
DNA	deoxyribonucleic acid
IEM	Illumina Experiment Manager
Indel	Insertion-deletion
LR	long range
LRM	Local Run Manager
NaOH	sodium hydroxide
NGS	next generation sequencing
PCR	polymerase chain reaction
SR	single read
RUO	research use only
SNV	single nucleotide variation
VAF	variant allele frequency

15 Revision history

Version 2025-07-01
Updated front page.

Version 2025-06-30
First edition.