ScisGo®

Chimerism v5

CHIM-24S-v5 Product Insert

Please read this product insert before use and keep for your convenience.

Overview

ScisGo® Chimerism Typing Kit v5 is designed for use on the Illumina® MiSeq™ Sequencing by Synthesis (SBS) platform to perform chimerism testing (engraftment analysis) on sample DNAs.

Included in Kit

① Stage 1 PCR – Reaction Plate……..……..……..……..……...10 µl per well (96 well plate)
② Stage 1 PCR – S1 Buffer……..……..……..……...1.5 ml (2 ml tube)
③ Stage 2 PCR – S2 Buffer……..……..……1.8 ml (2 ml tube)
④ Sequencing Primers – R1, Index, and R2……..……30 µl each (2 ml tube)

Not Included in Kit

① Platinum™ Taq DNA Polymerase (5 U/ µl)……..……Invitrogen™ (Cat. No. 10966018)
② Select-A size DNA Clean & Concentrator…..…….Zymo Research® (Cat. No. D4080)[a]
③ Quant-It™ PicoGreen® dsDNA Assay Kit……..Thermo Fisher Scientific® (Cat. No. P11496)[b]
④ MiSeq™ Reagent Kit…….....Illumina® (Cat. No. MS-102-2001, MS-102-2002, or MS-102-3001)
⑤ 2N Sodium Hydroxide (2N NaOH)……..…….....J.T.Baker™ (Cat. No. 02-004-137)[c]

[a]Alternative size selection kits are applicable, e.g. Qiagen® GeneRead *Size Selection* Kit (Cat. No. 180514).
[b]Alternative quantification methods are applicable, e.g. Qubit™ dsDNA HS Assay Kit (Cat. No. Q32854).
[c]Alternative molecular grade NaOH applicable, e.g. Honeywell® Fluka™ NaOH (2N) (Cat. No. 35254-1L).

Equipment Requirements

① Single- and multi-channel pipettes ranging from 2 µl to 1000 µl
② 96-well thermocycler (e.g. GeneAmp® 9700)
③ Plate centrifuge, microcentrifuge, and vortexer
④ Agarose gel electrophoresis apparatus (optional)
⑤ Flourometer (e.g. BioTek® Synergy™ or Qubit®)
⑥ Illumina® MiSeq® Instrument

For Research Use Only. Not for use in diagnostic procedures.

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Directions for Use

I. Stage 1 PCR

A. Thaw S1 Buffer at RT and thoroughly vortex before use.
B. Thaw Reaction Plate at RT, gently vortex, then spin down at 1400-2000 rpm for at least 1 min before removing well-caps.
C. Transfer 2 µl of DNA (minimum ~1 ng/µl) to the corresponding amplicon wells and spin down.
D. Add 19 µl of Platinum™ Taq to the S1 Buffer tube and gently vortex.
E. Add 8 µl of the S1 Buffer and Platinum™ Taq mix to each well and spin down.
F. Seal Reaction Plate and run the following thermocycling routine:

Stage 1 PCR Routine

95 °C	→	95 °C	→	60 °C	→	72 °C	→	4 °C
15 min		30 sec		30 sec		1 min		Hold

20 cycles

II. Stage 2 PCR

A. Remove Reaction Plate from thermocycler and spin down at 1400-2000 rpm for at least 1 min before removing well-caps.
B. Thaw S2 Buffer immediately before use. After thawing, thoroughly vortex.
C. Add 10 µl of S2 Buffer to each corresponding well of Reaction Plate, then spin down. ***Do not transfer the PCR product to a new plate.***
D. Seal Reaction Plate and run the following thermocycling routine:

Stage 2 PCR Routine

37 °C	→	95 °C	→	95 °C	→	50 °C	→	72 °C	→	72 °C	→	4 °C
60 min		15 min		30 sec		30 sec		1 min		7 min		Hold

20 cycles

E. Proceed to Step III or store at -20°C for up to 6 months.

III. Reaction Check (Optional)

A. After Stage 2 PCR is complete, spin down Reaction Plate and remove 3 µl of amplicon product from several samples, e.g., 8 per amplicon, and visualize on a 1.5% agarose gel.
B. Proceed to Step IV or store at -20 °C for up to 6 months.

IV. Amplicon Sample Pooling

A. For each amplicon, transfer 20 µl from each reaction well into a microcentrifuge tube.
B. Thoroughly vortex each of the 4 amplicon pools and proceed to Step V. or store at – 20 °C for up to 6 months.

V. Clean Up and Size Selection of Amplicon Pools

Use 100 µl of each amplicon pool for purification.

- **Option 1:** Select-A size DNA Clean & Concentrator (Zymo Research® Cat. No. D4080)
  A. Prepare Zymo Size Selection Binding Buffer Mix (550 µl per sample) according to Table 1:

Table 1. Zymo Size Selection Binding Buffer Mix

Component	Zymo DNA Binding Buffer	Molecular Grade NaOH (2N)	Molecular Grade 100% Ethanol	*Total Volume*
Total (5 rxns)	2490 µl	10 µl	250 µl	*2750 µl*

  B. Follow Zymo guide steps 2-7 (elution volume; 40 µl).

- **Option 2:** GeneRead™ Size Selection Kit (Qiagen® Cat. No. 180514)
  A. Follow Qiagen® guide (Protocol: GeneRead Size Selection of DNA Libraries Prepared with the GeneRead™ DNA Library Prep I Kit) (elution volume; 30 µl).

- **Option 3:** 0.7X AMPure XP Magnetic SPRI Beads (Beckman Coulter® Cat. No. A63880)

Visualize product on a 1.5% agarose gel to confirm removal of bands < 200 bp.

VI. Reaction Product Quantification

- **Option 1:** Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific® Cat. No. Q32854)
  A. Measure standards: 10 µl (Standard #1, Standard #2) and 190 µl dye mix.
  B. Measure samples: 2 µl of each purified amplicon pool and 198 µl dye mix.
  C. Enter the measured value (ng/µl) into the “Quant and Pool Qubit HS” worksheet in the CHIM-v5-Pooling-Workbook.xlsx.

- **Option 2:** Quant-iT™ PicoGreen® dsDNA Kit (Thermo Fisher Scientific® Cat. No. P11496)
  A. Measure 2 µl of each purified amplicon pool and Lambda Standard (Ex/Em: 480/520 nm).
  B. Enter the measured value (RFU) into the “Quant and Pool Picogreen” worksheet in the CHIM-v5-Pooling-Workbook.xlsx.

- **Option 3:** User-defined method for quantitating DNA concentrations
  A. Enter the measured DNA concentration (ng/µl) to the “Quant and Pool Qubit HS” worksheet in the CHIM-v5-Pooling-Workbook.xlsx.

VII. Combine Quantified Pools

A. Using volumes given by the CHIM-v5-Pooling-Workbook.xlsx, combine the amplicon pools in a new 1.5 ml microcentrifuge tube.

B. Thoroughly vortex the pooled library and use the CHIM-v5-Pooling-Workbook.xlsx for MiSeq™ library dilution to calculate EB Buffer volume.

VIII. MiSeq Preparation and Loading

A. Follow the Illumina® MiSeq™ preparation instructions with the below parameters:

Step	Details
Dilute to 4 nM	5 µl pooled library See pooling template for EB Buffer volume
Denature	5 µl 4 nM library 5 µl 0.2N NaOH mix thoroughly
Incubate	5 min @ RT
Dilute denatured DNA	990 µl HT-1 Buffer 10 µl denatured library
Dilute to loading concentration (4 pM)*	200 µl 20 pM denatured library 800 µl HT-1 Buffer
Dilute MiSeq Primers	770 µl HT-1 Added directly to each Scis*Go*® R1, Index, and R2 vial
Add to tray	Port 17: add 600 µL of diluted library Port 18: add 600 µL of diluted R1 primer Port 19: add 600 µL of diluted Index primer Port 20: add 600 µL of diluted R2 primer

*Cluster density can be adjusted directly proportional to loading concentration.

B. Follow Illumina® guidelines to rinse and dry the flowcell.

C. Create a sample sheet using the MiSeq™ sample sheet template corresponding to the Scis*Go*® kit in use (CHIM-24S-v5).
  a. Fill in cells labeled “Investigator Name”, “Project Name”, and “Experiment Name”.
  b. Input sample ID names into cells under cell labeled “Sample_Name”.
  c. Click “save as” and use MS Number on MiSeq cartridge to name file (MSxxxxxxx-500V2).
  d. Save as .csv file.

D. Load the sample sheet on the MiSeq™ instrument.

E. Go to Illumina® MiSeq™ Control Software and follow the on-screen instructions to load the MiSeq™ and begin sequencing.

Note - Integrity of kit components is guaranteed for up to 6 months from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability. **Scisco Genetics** and **Scis*Go*** are registered trademarks of Scisco Genetics, Inc. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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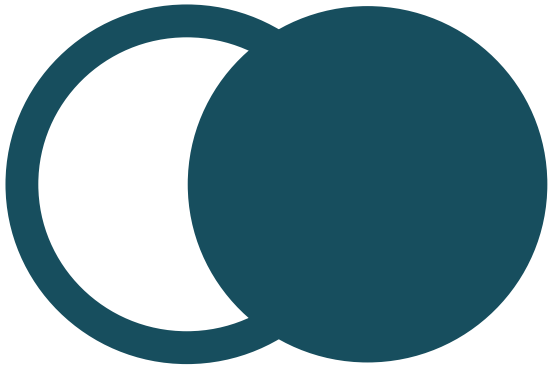
ScisGo® HLA v6

NGS Genotyping Kit (Cat. No. HLA-24S-v6)

Phased 3-field HLA typing in a single assay using proven Scisco Genetics technology and the GeMS-UI™ analysis software suite.

The human leukocyte antigens (HLA) are intimately involved in the innate and adaptive immune responses, communicating normal and abnormal cellular status to surveying immune cells. These highly polymorphic genes can greatly affect the outcome of hematopoietic cell and solid organ transplantation, in addition to their consequential roles in infectious disease, autoimmunity, and cancer.

Our Scis*Go*® HLA-v6 class I and II NGS typing assay is carried out via multiplex PCR of HLA specific amplicons. Exon and flanking intron sequences from the HLA genes are amplified in 4 reactions, simultaneously tagged with index sequences, pooled, and sequenced. Data can be stored and analyzed on the cloud and visualized on a desktop computer using our GeMS-UI™ (Genetics Management System) analysis software.



ScisGo® CHIM v5

NGS Genotyping Kit (Cat. No. CHIM-24S-v5)

Post-transplant analysis of the donor/recipient origin of white blood cells in peripheral blood and/or marrow.

A test for chimerism after allogeneic hematopoietic cell transplantation (HCT) is routinely performed as a prognostic measure of engraftment and related clinical outcomes. Chimerism testing employs methods commonly used in identity testing to distinguish and quantitate donor and recipient cells present in blood, bone marrow, and various tissues.

Our 4-reaction Scis*Go*® CHIM-v5 assay employs simple to perform laboratory steps and takes advantage of Next Generation Sequencing (NGS) technology to advance testing into the next generation of sensitivity and accuracy. The assay can be adjusted for capacity and sensitivity by varying the number of reactions used and samples analyzed. Turnaround times from DNA to data are comparable to other commonly used methodologies.

Genotyping Services

High-throughput – fast, accurate, affordable.

Using next generation sequencing technology, we provide fast, accurate, and affordable high-resolution HLA and KIR typing services. Our methods enable simultaneous processing of hundreds or thousands of samples, minimizing costs and turnaround times.

The HLA class I and class II typing system delivers 3-field typing for each gene in the 11-locus gene family.

Class I NGS sequence-based typing

HLA-A, B, C: exons 1-7

Class II NGS sequence-based typing

DRB1/3/4/5, DQA1, DQB1, DPA1, DPB1: exons 1-4

Using the structures of over 40 KIR gene content haplotypes, our KIR haplotyping assay can report unambiguous combinations of KIR haplotypes in an individual. Allelic typing is available for select KIR loci.

High-resolution typing the MICA and MICB genes, the Fc-gamma receptor gene family, and chimerism testing are also available.

Upgrade Now to NGS for Solid Organ Transplants

Compared with SSOP systems, NGS technology greatly reduces HLA typing ambiguities – yielding all loci at 3-field resolution – while maintaining SSOP levels for cost, sample manipulation and consumables.

- Both reagent costs and turnaround times from DNA to data are comparable between SSOP and Scis*Go*® HLA v6.
- High resolution typing provides greater confidence in patient/donor matching.
- High resolution allele assignments from NGS may be useful for patients with donor-reactive antibodies.
- Elimination of long and complex SSOP ambiguity codes simplifies reporting and clinical interpretation.
- An all-in-one solution to meet HCT and Solid Organ HLA typing needs.
- NGS is the logical, “next generation” choice for all HLA typing.