

**Fast and Reliable HLA Typing  
 on Precast PCR-CheckIT™ Gels**
**Ordering Information**

Product	P/N
PCR CheckIT™ Wide Mini S-4x25 with EtBr	<b>3304EB</b>
ORIGINS by Elchrom™ Electrophoresis System (including Catamarans S-8, S-12 & S-50/100, 50 ml of 40 x TAE running buffer stock solution, special forceps and instruction manual)	<b>2100 E</b>
Power Supply, 200 V/2000 mA, including timer	<b>2029 E</b>
Ethidium Bromide, 1mg/ml stock solution (50 ml)	<b>3030</b>
40 x TAE Running buffer (20 tubes of 50 ml)	<b>3031</b>
Easy Stain Gel Tray	<b>2344</b>
Bind-ET™ - Ethidium Removal System with cartridge	<b>2350</b>

Elchrom products are for research use only

PCR process is covered by US patents 4,683,195 and 4,683,202 owned by Hoffman-La Roche. Use of the process may require a license. SYBR is a trade mark of Molecular Probes.

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**Introduction**

The Human Leukocyte Antigen System (HLA) is part of the genetic region of the Major Histocompatibility Complex of humans. Proteins expressed by HLA are presented at the surface of cells. HLA proteins are highly diverse and unique to each person and allow the immune system to distinguish between self- and non-self cells. HLA plays a key role in the defence against pathogens, disease defence, predisposition to diseases, drug sensitivity, reproduction, cancer, autoimmunity.

The vital role of the HLA in the immune function has implications when transplanting organs. A close match between the HLAs of donor and recipient is imperative to promote grafting and to avoid post-transplant complications such as graft-versus-host-disease (Kaneku; Luque; Mao).

Transplantation success is highly correlated with the matching of six HLA markers. Testing HLA markers through PCR-based techniques is, thus, vital for tissue matching (Robinson; Zetterquist). Since 70% of the patients receiving transplants have no suitable donor in their family, diagnostic HLA testing is key to safeguard a transplantation by finding a perfectly matched donor (National Bone Marrow Program). Finding an HLA-compatible donor is a key to allogeneic transplantation of hematopoietic cells to treat hematologic malignancies, too (Demirer).

**SSO and SSP method**

There are two methods of choice for HLA kits: Sequence Specific Oligonucleotides (SSO) and Sequence Specific Primers (SSP). Both allow amplification of polymorphic target sequences in the HLA locus by the Polymerase chain reaction. Polymorphic HLA genes (such as e.g. HLA-A, B,

C, DP, DQ, and DR) can be routinely typed for in clinical HLA laboratories (Marsh et al; Zetterquist). If a cost-effective system is at hand, large populations of normal and affected individuals can be screened. Analysis of diagnostic bands through electrophoresis then allows matching transplant donors and recipients (Wordsworth; Erlich). Elchrom's Standard Operating Procedure defined PCR-CheckIT™ S-4x25 gels to be the most effective format to analyze a typical standard set of HLA markers per patient, amplified in 96-well plates.

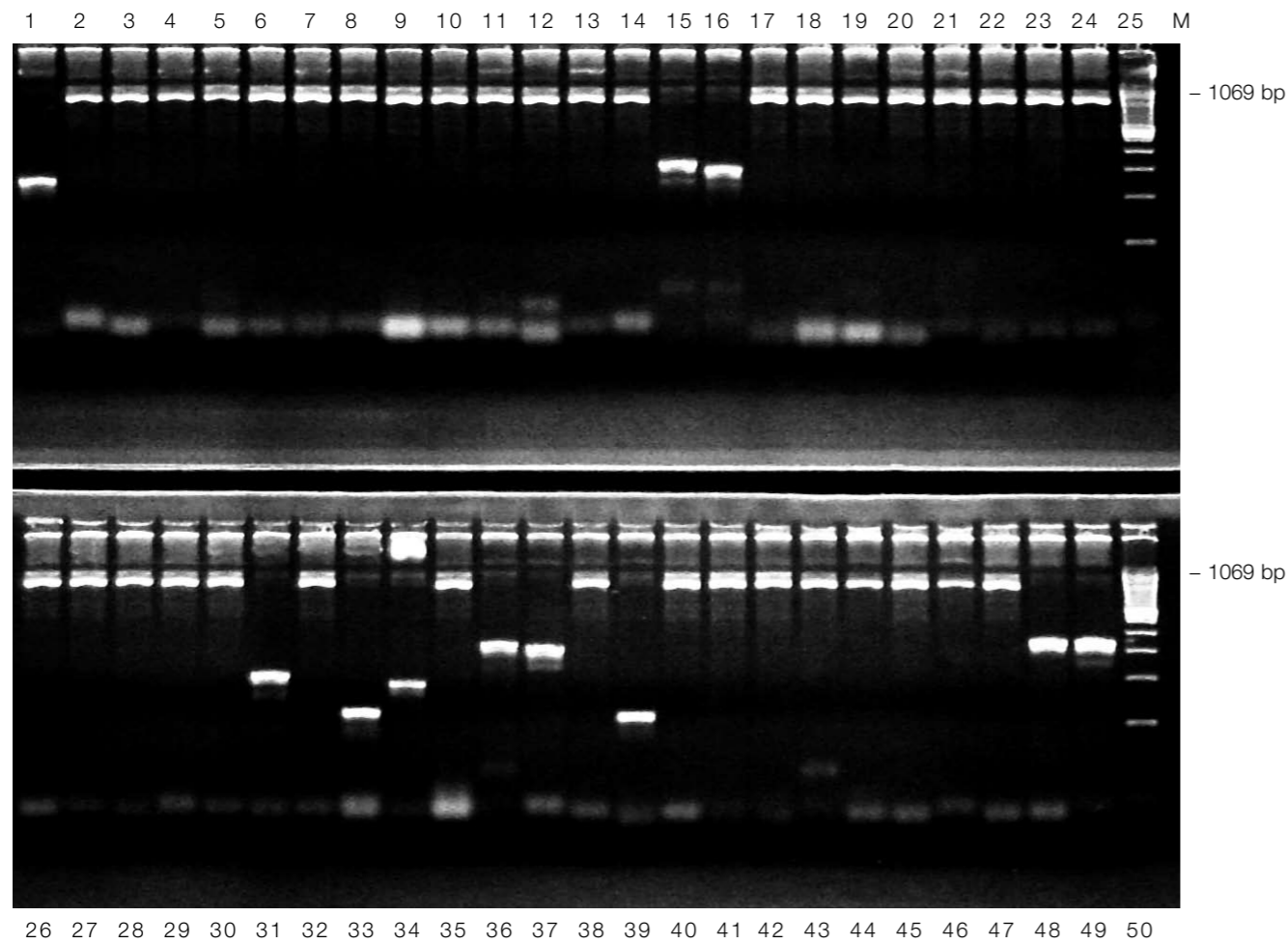
**Precast PCR CheckIT™ gels run in ORIGINS by Elchrom™ Electrophoresis System allows for:**

- **Fast results** - PCR analysis in as little as 7 minutes
- **Reproducibility - same quality of results gel after gel**
- **Easy handling** - ready-to-use gels, simple loading of samples
- **Reliability** - results of the highest fragment size accuracy
- **Stable gels** - mechanically and temperature stable gel with a shelf life of 18 months

**Experimental Procedure Equipment**

Samples were analysed by electrophoresis on a PCR CheckIT™ S-4x25 gel with EtBr. The PCR products were directly loaded on the gel using electronic equalizer pipette. Accurate identification of PCR products was easily done after run by standard HLA analysis software. Electrophoresis was performed in an ORIGINS by Elchrom™ Electrophoresis System at 12V/cm (144V). The temperature of the running buffer was kept constant at 20°C by using the internal temperature control system.

## HLA Typing



Analysis of HLA-B loci on a PCR CheckIT™ S-4x25 gel with EtBr. Top of the gel: Lanes 1, 15 and 16: specific PCR products. Lanes 2 to 14 and 17 to 24: negative for the specific amplifications. M: molecular marker 100 bp. Bottom of the gel: Lanes 31, 33, 34, 36, 37, 39, 48, 49: specific amplifications. HLA-B locus is typed as HLA-B\*07/\*51.

## Sample Preparation and Running Conditions

HLA fragments were amplified by PCR as described in User Manual of commercially available kits. 8 µl of sample was loaded onto the PCR CheckIT™ S-4x25 gel with EtBr. Electrophoresis was performed for 7 min at 144V and 20°C in 30 mM TAE running buffer containing EtBr at concentration of 0.5 µg/ml.

## Detection

Elchrom's PCR CheckIT™ S-4x25 were prestained with Ethidium Bromide and the results were visible immediately after electrophoresis by viewing the gel on UV transilluminator.

## References

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