University Clinic Heidelberg
Department of Transplantation Immunology
Im Neuenheimer Feld 305
D-69120 Heidelberg - Germany
Phone: +49-6221-564013
Fax: +49-6221-564200
www.ctstransplant.org

Manual No.:	**32**
Revision:	**July 11, 2017**
Product No.:	**502**
Lot No.:	**CYC20**

CTS *Collaborative Transplant Study*

WORKING INSTRUCTION

CTS - Cycler Control Kit

LOT-SPECIFIC MANUAL

To be applied to the following product:

Product No.	Description	
502	CTS - Cycler Control Kit	CE

Introduction

- **Attention:**
  All CTS-PCR-SSP Kits (for both, HLA class I and HLA class II typing) can be used with the new "Mastermix SSP" as PCR buffer. There is no change in the Mastermix volume to be used for setting up the PCR reaction. Taq polymerase is also required as previously.

- Intended use: The CTS-Cycler Control Kit provides a system for functional testing of the performance of thermal cyclers used for polymerase-chain reactions (PCR).

- Each well of the 96-well PCR tray contains a lyophilized mix of two primer pairs which amplify two fragments (90 base pairs and 515 base pairs). A PCR-buffer ("Mastermix") and a DNA sample are included in the kit. Setup and run the PCR in the thermal cycler which is to be tested.

- The PCR-products are then loaded onto a 2% agarose gel for electrophoresis. After electrophoresis, the ethidium bromide-stained gel is photographed and interpreted.

- If the temperature profile of the thermal cycler shows a deviation in one or several positions of heat block, amplification is less effective in the corresponding wells of the tray and the gel photography shows either amplicons of variable intensities or even no amplicons in certain slots.

- The CTS-Cycler Control Kit is specifically suitable to laboratories which use their thermal cyclers for HLA typing because this kit provides an overall-check of the efficiency of the thermal cycling conditions which are normally set up for HLA-typing by the PCR-SSP method.

- Additional general information on the CTS-PCR-SSP Tray Kits can be found in the “Working Instruction for the CTS-PCR-SSP Tray and Minitray Kits/Main Manual” (Manual No. 100A) and the “Material Safety Data Sheet for the CTS-PCR-SSP Tray and Minitray Kits” (Manual No. 100B) which are provided along with this kit. However, for specific PCR conditions applied to the CTS-Cycler Control Kit, please follow the instructions given in Section 2. of this Manual.

Table of contents

1. KIT COMPOSITION AND STORAGE CONDITIONS

- **Each** of the thin-walled, plastic, purple 96-well PCR trays contains prepipetted and lyophilized PCR primer mixes. The composition of the primer mixes is the same in all wells.
  Store at 2…8°C (in originally sealed pouch or after opening the package) until expiration date.

- **Number of 96-well trays (= number of tests for 96-well block cyclers) per kit:** 10

- **Cycler Control DNA:** 1 tube (540 µl)
  Store at -20…-60°C until expiration date.

- **Mastermix SSP** (PCR-Buffer without Taq-Polymerase): 1 tube (3 ml)
  Store at -20°C until expiration date. An aliquot which is thawed and has been opened for use can be stored at 2…8°C for 1 month.

2. PREPARATION OF THE PCR MIX

2.1 Thaw the frozen Mastermix
We strongly recommend to prepare small aliquots of Mastermix:
276 µl of Mastermix for one tray.
Freeze the aliquots and rethaw only the number of tubes needed.

2.2 Carefully remove one or more trays from the pouch. Start with the bottom tray. Reseal the pouch and place unused trays back into the refrigerator (2…8°C).

Besides the general use for 96-well thermal cyclers, the trays can be cut to appropriate size and used e. g. with 48- or 24-well thermal cyclers as well.

2.3 Place the tray inside a sample holder, such as a clear microtiter plate or a 0.2 ml tube rack (8x12), and place it in a clean area during sample setup.

2.4 Remove Taq DNA Polymerase from the freezer and keep it chilled during setup (e.g. on ice).

2.5 Add water and Taq DNA Polymerase to the aliquoted Mastermix and vortex thoroughly (see Table 1 for volumes of water and Taq).

2.6 Add the volume of Cycler Control-DNA to the Mastermix-Taq-water mixture as indicated in Table 1 and vortex thoroughly.

Table 1

Mastermix SSP (µl)	Aqua dest. (µl)	Taq DNA Polymerase at 5 U/µl (µl)	Cycler Control DNA (µl)	Total volume of PCR mix (µl)
276	**708**	**5.5 - 6.5***	**50**	**approx. 1039**

* The exact amount of Taq-Polymerase needed may vary according to the brand and lot number and therefore should be established through your own validation.

!!!** Please use the instructions given above for the correct set-up, **do not** use the amounts indicated in table 1 of the 'Working Instruction for the CTS-PCR-SSP Tray and Minitray Kits' (Manual No. 100A)**!!!

Using an electronic dispensing pipettor, dispense **10 µl** of the reaction mixture into each well. Be careful to dispense the drops onto the side walls near each well's top, allowing the dispensed drop to slide down. Do not allow the pipette tip to touch the bottom of wells.

Confirm that each well contains sample by noting the presence of the solution in each well. A settled sample will be indicated by a pink solution color on the bottom of wells. If a drop is hung up on the side of well, GENTLY tap the Tray/Minitray in the holder against the bench. Sufficient volume is supplied to allow for pipetting losses.

2.7 Cap the tray with strip caps provided (or commercially available foils for 96-well PCR trays). Ensure the caps completely seal the wells to prevent evaporation.

2.8 Place the tray in thermal cycler and begin thermal cycling (see section 3. Thermocycling profile).

Sections 2.1 through 2.8 must be performed efficiently fast to minimize the time between sample addition and initiation of thermal cycling. Prolonged incubation at room temperature may cause mispriming and non-specific PCR products!

3. THERMOCYCLING PROFILE

3.1 It is important to obtain appropriate ramp times and precise temperature control for optimal results.

3.2 The following thermal cycler profile is optimized and validated with the thermocyclers that are indicated in the Manual No. 100A, Section 2.6. If you use other cyclers, you may need to modify the profile or the ramp time for optimal band intensities.

Initial denaturation:	94°C, 2min
Denaturation:	94°C, 15 sec
Annealing+Extension:	65°C, 1 min
	10 cycles
	followed by
Denaturation:	94°C, 15 sec
Annealing:	61°C, 50 sec
Extension:	72°C, 30 sec
	20 cycles
Hold:	4°C, 15 min

3.3 After thermal cycling, remove the tray and proceed to gel electrophoresis.

4. GEL ELECTROPHORESIS

Absence or presence of PCR products is visualized by submarine agarose gel electrophoresis. Conditions are described for the Pharmacia cell GNA 200 (gel size 20x25 cm).

4.1 Preparation of the agarose gel

4.1.1 While the PCR is running, pour a 2% agarose gel. If you use the CTS electrophoresis chamber and the CTS combs, proceed as follows:

4.1.2 Add 7 g agarose and 7 ml 50x TAE buffer to 350 ml of ddH_20.

4.1.3 Boil to dissolve the agarose, using a magnetic stirring hotplate or a microwave oven.

4.1.4 Cool to 60°C, add 17 µl ethidium bromide, mix and pour the gel. Allow the gel to set for 1 hour at room temperature.

4.1.5 On a 20x25 cm gel you can place up to six CTS combs. These combs have a tooth distance corresponding to that of the channels of a standard 8-channel pipette. This allows the use of such a pipette for rapid loading of the samples onto the gel.

4.2 Electrophoresis

4.2.1 When the PCR is finished, remove the PCR Tray from the thermocycler.

4.2.2 Carefully remove the strip caps or the PCR-foil from the PCR Tray.

Caution: Sudden movement of the tray can disperse amplified product, contaminating the laboratory and may require repetition of the test!

4.2.3 Remove the combs and place the gel into the electrophoresis cell. The level of electrophoresis buffer should be 2 - 3 mm above the gel surface. The electrophoresis buffer can be reused several times.

4.2.4 Load 10 µl of each PCR product onto the gel. Because of the glycerol and the cresol red included in the mix, there is no need for use of any additional loading buffer.

4.2.5 Run the electrophoresis for 20 min at 170 V (approx. 0.4 V/cm^2).

4.2.6 Turn off power, disconnect electrodes and remove gel.

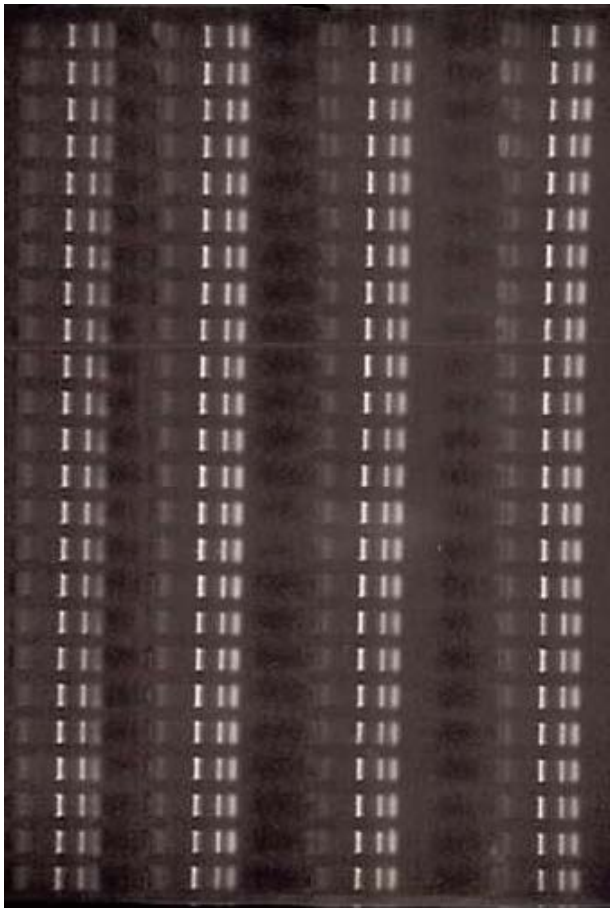
4.2.7 Place the gel on a UV light transilluminator (312 nm) and take a polaroid picture for interpretation and documentation.

5. RESULT EVALUATION

Two PCR fragments should be present in ***every*** slot: 90 base pairs and 515 base pairs. Pay also attention to equal intensity of all bands (see Figure 1).

In case of unequal or even absent amplification results in any slot, it is recommended to repeat the test. If the deficiency is reproducible, the thermal cycler needs to be serviced by the manufacturer.

Figure 1: Example of a Cycler Control test (96-well tray)



6. CONTACT

If you have any particular questions concerning this kit which are not answered in this or in the Main Manual, please do not hesitate to contact me or my co-workers at:

Tel.: ++49 6221 564013
Fax: ++49 6221 564200
E-mail: hien.tran@med.uni-heidelberg.de

Hien Tran, M.D.