EURx® MOLECULAR BIOLOGY PRODUCTS

HLA Taq DNA Polymerase HOT START

Cat. No.	size
E2717-01	200 units
E2717-04	500 units
E2717-02	1000 units
E2717-03	5000 units

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C. The reaction conditions are: 50 mM Tris-HCl (pH 9.0 at 25°C), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [^{3}H]dTTP), 10 µg activated calf thymus DNA and 0.1 mg/ml BSA in a final volume of 50 µl.

Storage Conditions: Store at -20°C.

Description:

- HLA Taq DNA Polymerase is a specially formulated enzyme for HLA SSP typing using commercially available kits.
- HLA Taq DNA Polymerase is a new generation “hot start” enzyme that is blocked at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during normal cycling conditions.
- Use of HLA Taq DNA Polymerase allows for the increase of PCR specificity, sensitivity and yield in comparison to the conventional PCR assembly method.
- Automatic “hot start” PCR is a fast and convenient method when assembling multiple PCR reactions.
- Both increased specificity and reduced mispriming improve multiplex PCR.
- Thermostable HLA Taq DNA Polymerase replicates DNA at 72°C and exhibits a half-life of 40 min at 95°C.
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- Contains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity.
- Adds extra A at the 3' ends.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and non-specific single- and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Volume of The Components Needed per Test Using Olerup SSP HLA Typing Kits without Taq DNA Polymerase:

No. of wells per test	Volume of Olerup Master Mix (µl)	Volume of DNA sample (µl)	Volume of H_2O (µl)	Volume of HLA Taq DNA Polymerase 5U/µl (µl)
8	30	20	49.2	0.8
16	54	36	88.6	1.4
24	81	54	132.8	2.2
32	108	72	177.1	2.9
48	162	108	265.7	4.3
96	312	208	511.7	8.3

Notes:

1. Mix all the components of PCR reaction according to the Olerup SSP HLA Typing Kits manual. Use HLA Taq DNA Polymerase in quantities recommended by Olerup Reference Table (see the exemplary table on the left side).
2. Total reaction volume in each well is 10 µl.
3. Use pure DNA samples in concentration of 15-30 ng/µl. DNA should not be resuspended in solutions containing EDTA in concentration above 0.5 mM.

Thermal Cycling Conditions with Olerup SSP HLA Typing Kits:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94°C	2 min	1
Denaturation	94°C	10 s	10
Annealing/ Extension	65°C	60 s	
Denaturation	94°C	10 s	20
Annealing	61°C	50 s	
Extension	72°C	30 s	
Cooling	4°C	Indefinite	1

Notes:

1. Program the cycler according to the Olerup SSP HLA Typing Kits manual.

Volume of The Components Needed per Test Using One Lambda Micro SSP HLA DNA Typing Trays without Taq DNA Polymerase:

No. of rows per test	No. of PCR reactions per test	Volume of D-mix (µl)	Volume of DNA (µl)	Volume of HLA Taq DNA Polymerase 5U/µl (µl)
1-2	1-16	180	19	1.2
3	17-24	270	29	1.8
4	25-32	360	39	2.4
5	33-40	450	49	3
6	41-48	540	59	3.6
7-8	49-64	720	79	4.8
9-12	65-96	1000	111	6.7

Notes:

1. Mix all the components of PCR reaction according to the One Lambda Micro SSP HLA DNA Typing Trays manual. Use HLA Taq DNA Polymerase in quantities recommended by the table on the left side.
2. Total reaction volume in each well is 10 µl.
3. Use pure DNA samples in concentration of 25-200 ng/µl (100 ng/µl is optimal). DNA should not be resuspended in solutions containing EDTA in concentration above 0.5 mM.

Thermal Cycling Conditions with One Lambda Micro SSP HLA DNA Typing Trays:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	96°C	2 min 10 s	1
Annealing/ Extension	63°C	60 s	
Denaturation	96°C	10 s	9
Annealing/ Extension	63°C	60 s	
Denaturation	96°C	10 s	20
Annealing	59°C	50 s	
Extension	72°C	30 s	
Cooling	4°C	Indefinite	1

Notes:

1. Program the cycler according to the One Lambda Micro SSP DNA Typing Trays manual.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Volume of The Components Needed per Test Using inno-train HLA-Ready Gene Kits without Taq DNA Polymerase:

Article	PCR tubes per typing	Volume of H_2O (6 µl)/well)	Volume of ReadyPCR (3 µl)/well)	Volume of HLA Taq DNA Polymerase 5U/µl (0.08 µl/well)	Volume of DNA sample (1 µl)/well)
A, DR, C	24	168	84	2.2	26
ABDR, ABC	96	624	312	8.3	102
DRDQ	32	222	111	3.0	35
B	48	324	162	4.3	52
DQ, B5/57 cross	8 + 1 Neg. Control	60	30	0.8	10
B27, B57, Narcolepsy	1 + 1 Neg. Control	12	6.0	0.2	1
Coeliac Disease	2 + 1 Neg. Control	24	12.0	0.32	2

Notes:

1. Mix all the components of PCR reaction according to the inno-train HLA-Ready Gene manual. Use HLA Taq DNA Polymerase in quantities recommended by inno-train Reference Table (see the exemplary table on the left side).
2. Total reaction volume in each well is 10 µl.
3. Use pure DNA samples in concentration of 25-100 ng/µl. DNA should not be resuspended in solutions containing EDTA in concentration above 0.5 mM.

Thermal Cycling Conditions with inno-train HLA –Ready Gene Kits:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	96°C	2 min	1
Denaturation	96°C	15 s	10
Annealing/ Extension	65°C	60 s	
Denaturation	96°C	15 s	20
Annealing	61°C	50 s	
Cooling	4°C	Indefinite	1

Notes:

1. Program the cycler according to the inno-train HLA-Ready Gene manual.

Volume of The Components Needed per Test Using Biorad HLA SSP Kits without Taq DNA Polymerase:

No. of wells per test	Volume of Biorad PCR cocktail (µl)	Volume of DNA sample (µl)	Volume of H_2O (µl)	Volume of HLA Taq DNA Polymerase 5U/µl (µl)
8	44	11	55	0.85
18	100	25	125	2
24	120	30	150	2.4
48	228	57	288	4.5
96	440	110	550	8.8

Notes:

1. Mix all the components of PCR reaction according to the Biorad HLA SSP manual. Use HLA Taq DNA Polymerase in quantities recommended by the table on the left side.
2. Total reaction volume in each well is 10 µl.
3. Use pure DNA samples in concentration of 100 ± 50 ng/µl. DNA should not be resuspended in solutions containing EDTA in concentration above 0.5 mM.

Thermal Cycling Conditions with Biorad HLA SSP Kits:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94°C	2 min	1
Denaturation	94°C	10 s	10
Annealing/ Extension	65°C	60 s	
Denaturation	94°C	10 s	20
Annealing	61°C	50 s	
Cooling	4°C	Indefinite	1

Notes:

1. Program the cycler according to the Biorad HLA SSP manual.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23