



Miltenyi Biotec

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## 1. Description

<b>Components</b>	<b>1×96-well ChimerXact PCR Plate:</b> lyophilized singleplex PCR Reaction Mix <b>1×0.5 mL PCR Resuspension Buffer (10×)</b>
<b>Capacity</b>	For 96 tests.
<b>Product format</b>	96-well plate containing lyophilized single ChimerXact Reaction Mixes.
<b>Storage</b>	The ChimerXact PCR Plate is best stored at -20 °C, and may be stored at 2–8 °C for up to 4 weeks protected from light in the original aluminium pouch. Store the Resuspension Buffer at 2–8 °C. The expiration date is indicated on the label.

### 1.1 Principle of the ChimerXact Kits

The ChimerXact Kits allow for a precise and reliable chimerism quantification by amplifying appropriate STRs in singleplex PCR reactions. Initial identification of the of suitable STR markers discriminating between donor and recipient should be done using the ChimerXplain Kit (# 130-095-794). For each STR that is assayed using the ChimerXplain Kit, singleplex PCR reactions (ChimerXact Kits 1–10, table 1) are available to monitor mixed chimerism after transplantation.

Using the selected ChimerXact Kit, singleplex PCR is performed and analysed by capillary electrophoreses. The STR alleles are then assigned to recipient or donor according to the length determined previously by using the ChimerXplain Kit. The relation of donor and recipient cells is calculated using the signal intensity of the alleles measured as peak height or peak area and is usually specified as percent chimerism.

# ChimerXact Kits 1–10

## Singleplex PCR analysis

ChimerXact Kit 1 (D10S2325)	130-095-770
ChimerXact Kit 2 (D12S391)	130-095-780
ChimerXact Kit 3 (P450CYP19)	130-095-833
ChimerXact Kit 4 (D2S1360)	130-095-788
ChimerXact Kit 5 (D9S1118)	130-095-785
ChimerXact Kit 6 (MYCL1)	130-095-832
ChimerXact Kit 7 (D7S1517)	130-095-792
ChimerXact Kit 8 (D11S554)	130-095-782
ChimerXact Kit 9 (D8S1132)	130-095-791
ChimerXact Kit 10 (SE33)	130-095-831

No.	Locus ID	Chromosome	Dye	Code	Length range
1	D10S2325	10	FL	B	162–213
2	D12S391	12	FL	B	213–269
3	P450CYP19	15	FL	B	314–464
4	D2S1360	2	Joe	G	200–273
5	D9S1118	9	FL	B	80–128
6	MYCL1	1	FL	B	156–225
7	D7S1517	7	Joe	G	164–212
8	D11S554	11	TMR	Y	166–253
9	D8S1132	8	TMR	Y	330–379
10	SE-33	6	TMR	Y	138–305

Table 1: Overview on STR loci covered by ChimerXact Kits 1–10.

## 2. Short protocol

The amounts and conditions given are based on extensive optimization and validation using an Applied Biosystem 310 Genetic analyzer. Parameters and DNA amounts might need to be adapted for other capillary sequencer models.

### 2.1 Genomic DNA isolation

1. Isolate genomic DNA by any protocol that yields high-purity DNA.
2. Dilute sample DNA in 10 mM Tris/HCl, pH 8 to a final concentration of 0.8 µg/µL.

### 2.2 Set up PCR experiment

1. Combine 2.2 ng genomic DNA and 2.5 µL 10× PCR Resuspension Buffer and adjust with water to a final volume of 25 µL.
2. Dissolve lyophilized ChimerXact PCR Mix using 25 µL PCR-Resuspension Buffer containing 2.2 ng genomic DNA.
3. Run PCR using the following program:

### Cycling conditions

LID=105 °C

T=95 °C	11 minutes		
T=96 °C	1 minute		
T=94 °C	30 seconds		} 9 cycles
T=60 °C	30 seconds	R (RAMP) = 1 °C/second	
T=70 °C	45 seconds	R (RAMP) = 1 °C/second	
T=90 °C	30 seconds		} 21 cycles
T=60 °C	30 seconds	R (RAMP) = 1 °C/second	
T=70 °C	45 seconds	R (RAMP) = 1 °C/second	
T=60 °C	30 minutes		
Hold 4 °C			

The thermal cycler profile is optimized for Eppendorf and MJ Research cyclers.

### 2.3 Capillary electrophoresis

1. Sample preparation:  
Electrophoresis mastermix: Mix 1.5 µL size standard and 23.5 µL Hi-Di™ formamide per sample. Add 1 µL of ChimerXact PCR product to one 25 µL mastermix aliquot. Denature samples at 95 °C for 3 minutes and snap cool.
2. Prepare a sample sheet:  
Prepare a GeneScan™ sample sheet for 4 dyes. Type sample name, mark size standard column, and enter sample information for all samples to be analyzed.
3. Prepare a GeneScan injection list:  
Set up a new injection list and select the appropriate sample sheet. For all samples select  
“GS STR POP4 (1 mL) A”  
“inj. Secs” to 5  
“inj. kV” to 15.0  
“Run kV” to 15.0  
“run °C” to 60  
“Run Time” to 30  
matrix file  
Optionally activate the autoanalysis checkbox.  
**▲ Note:** The injection parameters might have to be adapted depending on the signal intensities. Lowering the injection time or voltage will decrease the peak signals.  
Load the denatured samples onto the sample tray, close the doors, and start electrophoresis.

### 2.3 Data analysis

1. Set up analysis parameters:  
Define the analysis parameters for peak detection. When employing the GeneMapper® software use the microsatellite sizing-only application.
2. Set up a size standard:  
Assign sizes to the respective peaks of a representative run containing the size marker.
3. Set up a project for sample analysis:  
Define samples to be analyzed. Choose appropriate analysis parameters, a size standard, and a matrix for the analysis. Perform data analysis.

### 2.4 Select STR peaks

Display the electropherograms and the corresponding table of the ChimerXact samples. Select the peak(s) for each STR according to the dye and the sizes for recipient and donor as determined during the ChimerXplain analysis.

### 2.5 Chimerism quantification

$$\% \text{ Chimerism} = (D1+D2)/(D1+D2+R1+R2) * 100$$

D = Peak height (or area) of donor alleles

R = Peak height (or area) of recipient alleles

For heterozygous donors and recipients without overlapping alleles.

The complete and detailed user manual for the ChimerXplain Kit and the ChimerXact Kits can be downloaded at [www.miltenyibiotec.com/chimerism-analysis](http://www.miltenyibiotec.com/chimerism-analysis) or ordered free of charge using the order number 140-002-982.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

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