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Quick Guide for Light Cycler ® 480 Instrument

HISTO TYPE B*27 Q

Test kit for tissue typing of HLA alleles on a molecular genetic basis

Electronic instructions for use see www.bag-healthcare.com

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REF 728200 **HISTO TYPE B*27 Q**

For use on the Roche Light Cycler®480 System II

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Product description

The HISTO TYPE B*27 Q kit is used for the molecular genetic detection of HLA-B*27 alleles The HLA-B27 protein is a variant of the human leucocyte antigen-B (HLA-B). The HLA-B27 protein is associated with different autoimmune diseases (Bechterew's disease or Spondylitis ankylosans respectively, Reiter's disease, reactive arthritis) and is, therefore, used as part of the diagnostic procedure (1, 2). A positive HLA-B27 result is associated with a very high disease risk. Especially in case of unclear suspicion of M. Bechterew, a secured HLA-B*27 diagnosis provides an important contribution to the therapy of the patient. Around 3% to 6% of the people carrying the HLA-B*27 gene develop Spondylitis ankylosans and more than 90% of all patients with a seronegative arthritis are carrying this gene.
The **HISTO TYPE B*27 Q kit** covers all common HLA-B*27 subtypes. Moreover, the kit differentiates between the disease associated alleles and the subtypes HLA-B*27:06 or HLA-B*27:09, which are not associated with Spondylitis ankylosans (3).

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Test principle

The test is performed with genomic DNA as starting material. The DNA is amplified in a PCR with sequence-specific primers (SSP). The primers were specially developed for the selective amplification of the Exons 2 and 3 of the HLA-B*27 gene, which do only recognize the B*27 subtypes. The amplicons are detected with likewise gene locus specific fluorescent dye-labelled hydrolysis probes (TaqMan® probes), which increases the diagnostic sensitivity and specificity of the test compared to a conventional SSP.

If amplicons are present, the probes are hydrolyzed by the Taq polymerase and a fluorescence signal is generated that increases proportionally to the amount of the PCR product. The fluorescence signals are measured by the optical detection unit of the RT-PCR-Cycler.

The test is performed in a single PCR reaction that detects the internal positive control (human HBB gene), the disease-associated subtypes and the non-disease-associated subtypes with different fluorescent colours.

Kit contents of the HISTO TYPE B*27 Q kit

Components	Description	Storage conditions
230 µl Q Primermix B*27	ready to use, contains primers and probes	≤ -20°C
230 µl Q Mastermix	ready to use, contains dNTPs,Taq Polymerase, reaction buffer	
Instructions for use (IFU)		

Additionally required reagents and devices (not included in the kit)

- Reagents for DNA isolation (validated DNA isolation see IFU)
- Real-Time PCR-Cycler (validated cycler see IFU)
- RT-PCR reaction tubes with caps or foils (validated products see IFU)
- Aqua dest.
- Piston pipettes (0,5 – 1000 µl) and tips

Storage and stability

The kits are shipped at 2...8°C. Upon receipt store all reagents in temperature monitored devices at ≤ -20 °C. The expiry date is indicated on the label of each reagent. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. The freeze-thaw cycle testing has shown that up to 15 cycles has no detrimental effects on the quality of the kit.

Test procedure

Safety conditions and special remarks

Molecular genetic techniques are particularly sensitive and should be performed by well trained personnel experienced in molecular genetic techniques. The results of these tests must not be used as sole basis for clinical decisions.

Special safety conditions must be observed in order to avoid contamination and thus false reactions:

- ♦ Wear gloves during work (powder-free, if possible).
- ♦ Use new tips with each pipetting step (with integrated filter).
- ♦ Use separate working areas for pre-amplification (DNA isolation and PCR set up) and post-amplification (detection). Preferably, use two separate rooms.
- ♦ Use devices and other materials only at the respective places and do not exchange them.

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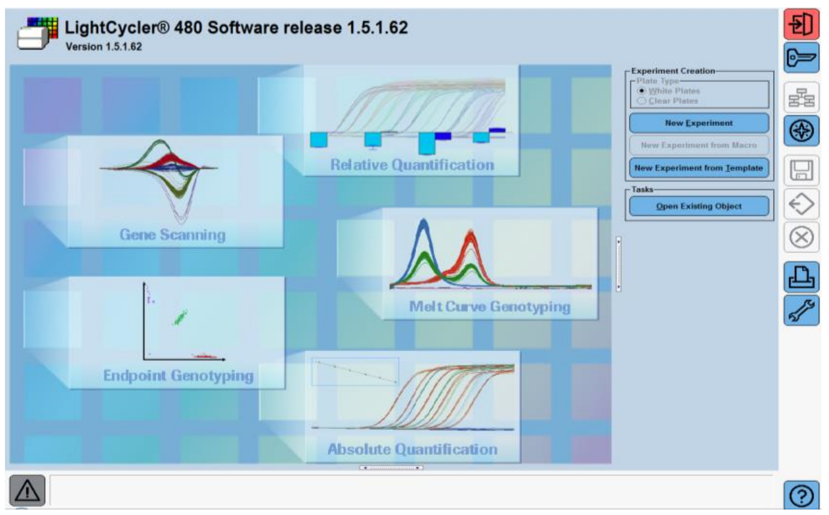
Due to overlap of the emission spectra of the dyes, one filter combination may pick up signals from a dye measured by another channel, a phenomenon called "crosstalk". Although each emission filter is optimized for a specific emission maximum, all fluorescent dyes currently available have emission spectra with long "tails," leading to this spectral overlap. This bleed-over of fluorescence signal can result in misinterpretation of data. To correct the crosstalk, color compensation (CC) can be applied before data analysis.

- ♦ A CC object can only be applied to experiments that were run on the same Light Cycler® 480 Instrument it was created on.
- ♦ Instead of running a separate color compensation experiment, you can also run the color compensation reactions in parallel to your experimental samples. In this case, apply the appropriate experimental PCR protocol, but always add a temperature gradient or melting curves program.
- ♦ For further information, please see the **IFU for Color Compensation** or refer to the LC®480 Instruments Operator's Manual, Software version 1.5, section Advanced Software Functionalities, Color Compensation Analysis.

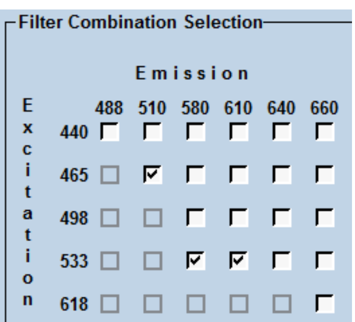
1. Getting started

- Start LC 480 Cycler
- Turn on the controle unit
- Log on to Windows
- Start the LightCycler® 480 software by double-clicking the <LightCycler480> icon.
- Enter the username and assigned password to log in to the LightCycler® 480 software.
- The overview screen displays. This screen allows entry of a new experiment with or without use of a template for the conditions, or run a previously programed macro.



Before creating a new experiment for HISTO TYPE B*27 Q the filter combination should be set. If the format has already been generated, continue to step 1.1.

- Go to tools ->
- Select “Detection Formats and click on “New”
- Name your detection formats (e.g. HISTO TYPE B*27Q)
- Set filter combination as follows:



- Change the names in the filter combination list as follows:
  -> The Melt/Quant Factors and the Max. Integration Time should be set as default.

Selected Filter Combination List

Excitation Filter	Emission Filter	Name	Melt Factor	Quant Factor	Max Integration Time (Sec)
533	580	Yakima Yellow	1	1	1
465	510	FAM	1	1	1
533	610	Texas Red	1	1	1

1.1 Create a new experiment.

- Go to “Overview” window ->
- Click on “ New Experiment”
- In “Experiment”/”Setup” select your “detection format” (HISTO TYPE B*27Q)
- Click “Customize” and make sure all three filter combinations are active (465-510; 533-580; 533-610) and the “Integration Time Mode” is set to “Dynamic”
- Set the reaction volume to 10 µl
- Set the PCR program as follows:

Program Name	Cycles	Analysis Mode	Target (°C)	Acquis. Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquis. (per °C)
Initial activation	1	None	96	None	00:10:00	4,4	-
Amplification	40	Quantification	96	None	00:00:20	4,4	-
			64	Single	00:00:40	2,2	-
Cooling	1	None	37	None	00:00:30	2,2	-

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- Save as template for speed up the process of creating a new experiment trough “New Experiment from Template” in the Overview window.
- Set your samples in “Sample Editor”
- Click “Start Run” in “Experiment”

1.2 Set up the “Subset Editor”

- Click on “ Subset Editor”
- Depending on your sample amount create a new “ID” (< 96 samples) in “Subsets” with ⊕ or take “All Samples”
- Click on “Apply” and go to “Sample Editor”

1.3 Set up the “Sample Editor”

- In “Step1: Select Workflow” select “Abs Quant”
- In “Step2: Select Samples” select in “Subset” your experiment name or “All Samples”
- Make sure all three filter combinations in “Select Filter Combinations” are active (465-510; 533-580; 533-610)
- Set the sample names (if required) and your quantification sample type for each position and filter combination.

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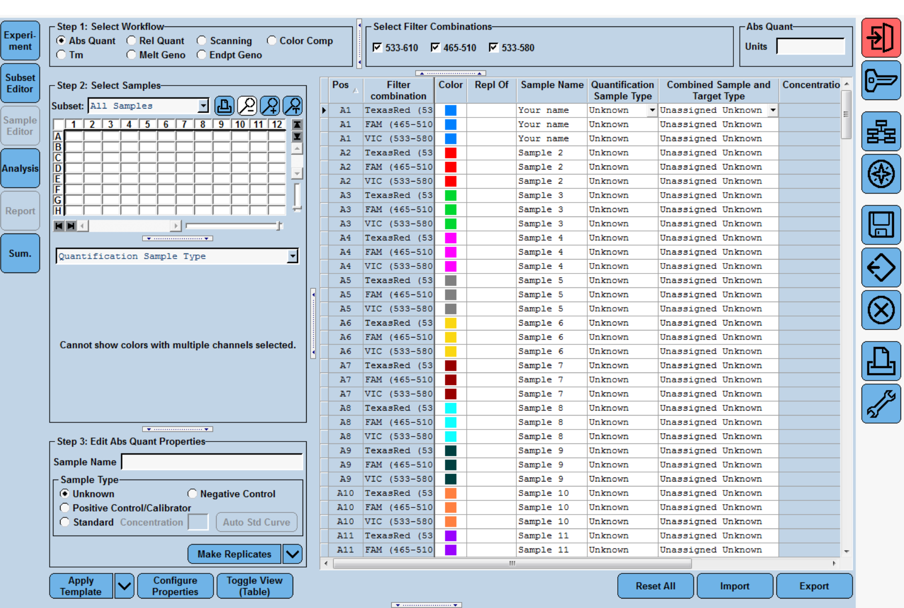

1.4 Prepare the reaction mix

For each sample the following reagents are pipetted into a reaction tube:

- 2 µl Q Primermix
- 2 µl Q Mastermix
- 1 µl Sample DNA (10-150 ng/µl)
- 5 µl Aqua dest.

The reaction volume for each Q-PCR test is 10 µl.

If a premix of Q Primermix, Q Mastermix and Aqua dest. is prepared for more than one sample please allow for a reasonable additional amount for pipetting losses.

If a negative control (NTC) should be performed prepare a PCR reaction with aqua dest. instead of DNA.

- After preparing and sealing the 96-well plate spin down the plate and set into the LC 480.
- Click “Start Run” in “Experiment”

2. Data analysis

- After performing the HISTO TYPE B*27 Q test kit go to “Analysis” and choose first the color comp object (CC-HT-B*27Q) from “In Database”.
- Click “Calculate” for each “Filter Comb” to get Cp results.
- Save your experimental data.

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TROUBLESHOOTING

Symptom	Possible reason	Potential solution
Bad or no signal	Presence of an inhibitor.	Use fresh B27 Q reagents.
	No gDNA in the reaction.	Repeat test. Take care of correct pipetting.
	Wrong amplification parameters.	Check PCR program and ramp rate.
	Contaminated or degraded DNA.	Check DNA concentration and quality. Check DNA on a gel. Repeat DNA isolation.
	Fluorescent probes or primers degraded.	Use fresh Q Primermix. Avoid exposition to light and frequent thawing and freezing. Observe storage conditions!
	Bubbles in the PCR reaction / remaining liquid at the inner wall of the tube.	Careful pipetting. Spin down PCR plate.
	Incompatible or low quality qPCR plastic ware.	Use compatible and high quality plastic ware (see chapter 3.3 IFU).
	Evaporation of the reagents due to incorrect closing of the PCR tubes.	Make sure that the PCR tubes are closed properly. Be careful at the edges of sealing foils.
Signal in the negative control	Contamination with DNA in the negative control.	Repeat the negative control. Decontaminate the workplace.

EXPLANATION OF SYMBOLS USED ON THE LABELS

	Sufficient for n tests
	Storage temperature / Lower limit of temperature
	Use by
	Consult instructions for use
	Manufacturer
HLA TYPING	Intended use: HLA typing
IFU	Instructions for use
RUO	For research use only
LOT	Batch code
Q Primermix | B27	Primermix for typing HLA-B*27 with the HISTO TYPE B*27 Q kit
Q Mastermix	Mastermix for the HISTO TYPE B*27 Q kit
REF	Catalogue number

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Technical assistance

http://service.bag-healthcare.com or phone +49 (0)6404-925-125

Instructions for use in other languages see http://www.bag-healthcare.com