OLERUP QTYPE®11 INSTRUCTIONS FOR USE

For Research Use Only. Not for use in diagnostic procedures.

Olerup QTYPE® HLA 11 Typing Kits - Instructions for Use (for Roche LightCycler® 480 II, ABI QuantStudio™ 6 Flex and Pro/7 Flex and Pro/Dx and ViiA™ 7)

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PRODUCT DESCRIPTION

Olerup QTYPE 11 HLA Typing Kits are *in vitro* tests for the DNA typing of HLA Class I and Class II alleles.

To be used as an aid in determining HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1 and/or DPB1 alleles with low to intermediate resolution in human genomic DNA samples extracted from anticoagulated blood.

The product is designed for use with the Roche LightCycler® 480 II or the Applied Biosystems QuantStudio™ 6 Flex and Pro/7 Flex and Pro/Dx and ViiA™-7 real time PCR instruments and SCORE™6 Interpretation Software.

The Olerup QTYPE 11 kit is for professional use by laboratory technicians trained in molecular biology techniques, working in histocompatibility and immunogenetics laboratories.

SUMMARY, EXPLANATION AND PRINCIPLES OF THE TEST

Olerup QTYPE 11 kits combine the principles of sequence specific primers (PCR-SSP) with hydrolysis probes for the amplification and real time detection of HLA alleles[1].

The PCR-SSP methodology is based on the principle that completely or almost completely matched oligonucleotide primers without 3' end mismatches are more efficiently used in the PCR reaction than mismatched primers by non-proofreading, thermo-stable DNA polymerases. Primer pairs are designed to be matched with single alleles or group(s) of alleles depending upon the degree of typing resolution required. With strictly controlled PCR conditions, matched or almost completely matched primer pairs allow amplification to occur, i.e. a positive result, whereas mismatched primer pairs do not allow amplification to occur, i.e. a negative result[2, 3].

In traditional PCR-SSP, the amplification products are detected using an agarose electrophoresis gel. Olerup QTYPE products differ in that each reaction also includes at least one hydrolysis probe, designed to bind to a location between the primers. Like primers, hydrolysis probes are also sequence-specific oligonucleotides. These probes differ from primers in that they are dual-labelled with a fluorescent reporting dye conjugated to the 5' end and a quenching molecule conjugated to the 3' ends. PCR amplification cannot occur from hydrolysis probes, even when fully bound to single stranded target DNA (ssDNA).

When the fluorescent reporter dye is excited with light at a specific wavelength it will emit light at a different wavelength. However, when the dye is in proximity to the quenching molecule, fluorescence energy resonance transfer (FRET) occurs, whereby the emission energy from the reporter dye is absorbed by the quenching molecule. Therefore, when hydrolysis probes are intact, very little light emission can be detected from the reporter dyes[4].

The real time PCR protocol is very similar to traditional PCR. Each cycle begins with a denaturation step where the reaction is heated to a temperature at which double stranded DNA (dsDNA) dissociates into single strands. During the annealing and extension stage the primers and probes will bind to the target ssDNA, assuming that they are complimentary. Taq polymerase binds to the 3' end of the bound primers and begins to incorporate dNTPs in order to extend the primer and create a new DNA strand which is complimentary to the target. When Taq polymerase reaches an area of dsDNA, for example an upstream location where a hydrolysis probe is bound, its 5' nuclease activity will degrade the probe. When a probe is degraded its reporter dye and quenching molecule are released from each other

into the reaction solution. With each cycle of the PCR more template is produced, meaning that more probe can bind and be degraded by the Taq polymerase. Therefore, the amount of free reporter dye increases in proportion to the amount of PCR product that is created.

Real time PCR is performed on a thermal cycler that includes an imaging unit consisting of a light source, excitation and emission filters, and a digital camera to image the PCR plate. At the end of each extension step of the PCR, the instrument illuminates the entire plate with light within a narrow wavelength range via the excitation filter. Light emitted from the free reporter dye is then detected by the digital camera, via an emission filter. Different filter combinations can be used to detect different reporter dyes, and each dye is imaged separately at the end of every cycle. For each filter combination a graph can be plotted showing the change in fluorescence against the cycle number.

Since real time PCR instruments have narrow band wavelength filters, multiple reporter dyes can be used in each PCR well. Therefore, a single PCR well may contain multiple sets of PCR-SSP primers, each with their own hydrolysis probe with a unique reporter dye, in effect allowing multiple independent reactions to occur and be detected simultaneously in a single well.

An advantage of hydrolysis probe-based PCR is that reactions can be designed with narrower specificities than traditional PCR-SSPs. This is due to the fact that the specificity of the reaction is not just dependent on the two *cis*-located priming sites, but also a third *cis*-located site where the probe binds. Olerup QTYPE 11 kits exploits this feature in order to increase the typing resolution of the kit.

Olerup QTYPE 11 kit utilises two functions of the amplification curve from each reaction in order to determine whether the reaction has given a positive result with the tested DNA sample: the C_q value (the point at which exponential amplification is detected) and the overall fluorescent value during the final cycle. The values from the HLA assays are normalised to the values from the internal amplification control assay in the same well, in order to account for any variances in DNA quality and concentration, or even minor pipetting errors.

REAGENTS AND MATERIALS

Identification

Olerup QTYPE 11 kits consist of a PCR plate containing pre-aliquoted and dried reaction mixes in each well. These reaction mixes consist of minimally one HLA assay and one internal control assay. Depending upon the level of multiplexing, there may be more than one HLA assay per well. Each assay within a single well uses a unique reporter dye. Each assay minimally consists of two PCR-SSP primers and a dual labelled hydrolysis probe. The wells are covered with a self-adhesive aluminium sealing sheet.

Each test is provided with a pre-aliquoted vial of master mix, complete with Taq polymerase. An optically-clear PCR sealing sheet is also provided for each test.

Each lot of Olerup QTYPE 11 kit comes with a lot-specific information sheet. The lot information can be found on the kit labels and is printed on to the PCR plate. Further lot-specific information can be found in SCORE 6. Refer to page 8 for more information on SCORE 6.

Test Controls

Each well contains an internal amplification control assay, designed to amplify and detect a conserved region of the human genome in the human growth hormone gene. In cases where the HLA assay(s) in a well are negative with the tested sample, this control is used to confirm that reaction mixture was correctly added to the well and that PCR occurred.

Each test plate includes several replicates of positive control wells for each fluorescent channel. These wells are designed to amplify and detect a conserved region of the human genome in the human growth hormone gene. SCORE 6 analyses these wells to ensure that the expected Cq, fluorescent strength and uniformity requirements are met. Refer to SCORE 6 Instructions for use for further details.

Each test also has a 'no amplification control' well (i.e. a negative control), to which molecular biology grade water is added instead of DNA. This is used to ensure that there is no DNA contamination in the master mix, molecular biology grade water or laboratory equipment used for the test.

Refer to Quality Control and Run Validity on page 10 for further information on the test controls.

Warnings and Precautions

1. For Research Use Only. The performance characteristics of this product have not been established.
2. Olerup QTYPE 11 HLA Typing Kits are intended as an aid to determine HLA genotypes. Because of the complexities of the HLA allelic definitions and primer/probe dense assays, an appropriately trained and qualified HLA professional should review and interpret the data.
3. Many HLA alleles do not have full sequence information published, particularly outside of exons 2 and 3 for Class I alleles, outside of exon 2 for Class II alleles, and for introns. In these instances, it is not known whether some of the primers or probes of the Olerup QTYPE 11 reactions are completely matched with the target sequences or not. It is assumed that unknown sequences in these regions are conserved within allelic groups.
4. Some Olerup QTYPE 11 reactions are designed to amplify rare alleles. In some cases, no positive DNA samples are available to test these mixes and they have therefore only been proven to be correctly negative with mismatched alleles. The lot-specific information sheet lists which mixes have not been positively tested for this reason.
5. Some Olerup QTYPE 11 reactions are designed to target rare sequence motifs in addition to more common and therefore testable motifs. In some cases, no DNA samples are available to confirm the reactivity. For these cases data from similar motifs that are possible to test are used as basis to define the reactivity.
6. Due to high similarity between classical and non-classical HLA genes, occasionally Olerup QTYPE 11 reaction mixes might amplify genes other than the intended 11 HLA loci, including HLA pseudogenes. Lack of full sequence information for the non-classical HLA genes and pseudogenes does not allow to fully assess the risk of this happening. Olerup QTYPE 11 and SCORE 6 interpretation software are not intended to interpret results for non-classical HLA genes and pseudogenes amplification.

7. Olerup QTYPE 11 HLA Typing kit should not be used for analysis of chimeric samples, i.e. samples that contain genome from more than one individual, e.g., either through mixing two genomic DNA materials, or samples obtained from individuals that underwent hematopoietic stem cell transplant.
8. *Biohazard Warning*: All blood products should be treated as potentially infectious. No known test method(s) can offer assurance that products derived from human blood will not transmit infectious agents. Handle all samples as if capable of transmitting disease. All work should be performed wearing gloves and appropriate protection.
9. Whilst Olerup QTYPE 11 kits can be considered a closed system as they do not require post-PCR manipulation, general PCR laboratory contamination precautions should still be observed, particularly if other HLA genotyping tests are carried out in the same facility. Pipettes and other equipment used for post-PCR manipulations should not be used for pre-PCR manipulations. Pre- and post-PCR activities should be conducted in different areas.
10. Do not use PCR plates that show obvious signs of physical damage such as cracks in the plastic or deformation of the upper rim of the wells, as these may lead to evaporation during PCR.
11. Do not use PCR plates where there has been obvious damage to the aluminium sealing sheet.
12. Use molecular biology grade water, certified free from DNase and RNase.
13. Use mixer tubes suitable for molecular biology, certified free from DNase and RNase.
14. Use suitable polystyrene reagent reservoir without any type of hydrophilic treatment/coating.
15. A relative centrifugal force of a minimum of 1000 rcf is required. Use the “rcf” setting of your centrifuge to determine the required revolutions per minute (rpm) of your specific centrifuge model. Refer to the instructions for use of your centrifuge if unsure how to set a relative centrifugal force.
16. Always centrifuge Olerup QTYPE 11 trays and Colour Compensation trays with a support, preventing attaching debris from the centrifuge to the outside of the wells and carrying them over to the real time instrument block. Any dirt in a heat block might result in a non-uniform heating and might cause Olerup QTYPE 11 reaction to fail.

Storage Instructions

Store the boxed kits in their original packaging in the dark at the temperatures indicated on the kit label.

Note that hydrolysis probes are light-sensitive, and it is therefore imperative that the correct storage conditions are used.

Use before the expiration date printed on the kit label.

Do not thaw the Olerup QTYPE 11 plates or master mix until use.

Materials Provided

1. Olerup QTYPE 11 HLA Typing Plates.

2. Master mix including Taq polymerase. Refer to the kit lot-specific information sheet for information on the master mix volumes.
3. Optically-clear adhesive sealing sheets.

Materials Required but Not Provided

1. DNA isolation kit and equipment.
2. Molecular biology grade water.
3. Instrumentation for determining DNA concentration and purity ($OD_{260/280}$).
4. Pipetting devices (suitable for 2, 10, 200 and 1,000 µl, multi-channel pipette device for 10 µl).
5. Suitable reagent reservoir.
6. Disposable pipette tips with filter barriers for all pipetting devices (2, 10, 200 and 1,000 µl).
7. Vortex mixer.
8. Mixer tubes.
9. Microcentrifuge.
10. Centrifuge with adapters for PCR plates (at a minimum of 1000 rcf).
11. Valid real time PCR instrument, see below.
12. PCR Seal Applicator.

Download of Lot Specific Information

1. 1. Go to www.caredx.com and find QTYPE under Products and Services - Transplant lab products - HLA typing solutions
2. Click on “QTYPE technical documents” and sign in
3. Click on “Product inserts”
4. Click on the Lot No you want to access
5. Lot Specific Information will open and is ready for printing on your local printer
6. Alternatively, use the search function to find all lot specific documents by searching with a specific lot number

INSTRUMENT REQUIREMENTS

Olerup QTYPE 11 kits are intended for use with the following instruments:

- Roche LightCycler 480 II real time PCR instrument with 384 silver block running LightCycler software version 1.5.x.x.
- Applied Biosystems QuantStudio 6 Flex and 7 Flex real time PCR instruments running QuantStudio software v1.x, with 384-well PCR block.
- Applied Biosystems QuantStudio Dx real time PCR instrument running QuantStudio Test Development Software v1.0.1, with 384-well PCR block.

- Applied Biosystems ViiA-7 real time PCR instrument running QuantStudio software v1.x or the ViiA-7 software v1.2.x, with 384-well PCR block.
- Applied Biosystems QuantStudio 6 Pro and 7 Pro real time PCR instruments running Design & Analysis Software 2.6.x, with 384-well PCR block.

The real time Instrument should be calibrated according to the accreditation rules provided by the American Society of Histocompatibility and Immunogenetics (ASHI) and the Clinical Laboratory Improvement Amendments (CLIA) instrument calibration standards.

Refer to the Instrument suppliers Instructions for Use for additional information.

SOFTWARE REQUIREMENTS

Olerup QTYPE 11 HLA Typing Kits are validated for use with SCORE 6 version 6.1 or later. SCORE 6 is a software designed to assist the clinician in determining the HLA type of samples tested with Olerup QTYPE 11 kits.

Refer to the SCORE 6 Instructions for use for information on hardware and operating system requirements.

Ensure that Roche LightCycler Software v1.5.x.x is used to operate the LightCycler 480 II real time PCR instrument.

Ensure that QuantStudio software v1.x is used to operate the Applied Biosystems QuantStudio 6 Flex/7 Flex real time PCR instruments.

Ensure that QuantStudio Test Development Software v1.0.1 is used to operate the Applied Biosystems QuantStudio Dx real time PCR instrument.

Ensure that the ViiA 7 software v1.2.x or the QuantStudio software v1.x is used to operate the ViiA 7 real time PCR instrument.

Ensure that Design & Analysis Software 2.6.x is used to operate the Applied Biosystems QuantStudio 6 Pro/7 Pro real time PCR instruments.

SPECIMEN COLLECTION AND SAMPLE PREPARATION

Samples that are tested with Olerup QTYPE 11 kit must be human genomic DNA extracted from anticoagulated blood. Olerup QTYPE 11 has been validated for use with these three extraction methods:

1. EZ1® DNA Blood Kit, using automated GenoM6, Genovision or EZ1 Advanced XL, QIAGEN
2. QIAamp® DNA Blood Mini kit, spin columns, QIAGEN
3. Gentra®PureGene® Blood kit, QIAGEN

Ensure that the blood samples are not heparinized, as this may interfere with the PCR and lead to incorrect results. Olerup QTYPE 11 has been validated with anticoagulated blood stored for up to five days at room temperature.

It is recommended that 10 ng of purified genomic DNA is used per reaction well, though Olerup QTYPE 11 has been verified with 10-50 ng DNA per reaction well. All DNA samples

should be measured by UV spectrophotometry and have an $OD_{260/280}$ ratio between 1.67 and 2.0.

DNA should be resuspended in molecular biology grade water. If a buffer containing EDTA is used, ensure that the total EDTA concentration does not exceed 0.5mM.

DNA samples may be used immediately after extraction or stored at +4°C to +8°C for up to 2 weeks with no adverse effects on results. DNA samples can be stored at -20 °C or colder for 9 months [5].

The purity and concentration of extracted DNA should be tested for acceptability prior to HLA typing. The following levels of common PCR interferants can affect the performance of Olerup QTYPE 11 kits. Ensure that the final 10 µl reaction mix does not contain levels of these substances exceeding these limits.

Interfering substance (units)	Accepted concentration
Bilirubin (µM)	0.625
Hemoglobin (mg/l)	9.375
Protein (albumin) (g/l)	0.391
Triglycerides (mM)	37
Citrate (% v/v)	0.25
EDTA (mM)	0.375

PROCEDURE

Before starting an Olerup QTYPE 11 test ensure to follow the instrument set-up procedure and that a valid Colour Compensation run has been performed on the real time PCR Instrument that will be used.

Refer to the following appendices for details on how set up the real time PCR instrument and how to perform a colour compensation run.

Roche LightCycler 480 II:

Appendix 1 – LightCycler 480 II; Colour Compensation

Appendix 2 – LightCycler 480 II; Run Protocol

ABI QuantStudio 6 Flex/7 Flex/Dx and ViiA-7:

Appendix 3 – ABI QuantStudio™ 6 Flex/7 Flex/Dx and ViiA-7; Colour Compensation

Appendix 4 – ABI QuantStudio™ 6 Flex/7 flex/Dx and ViiA-7; Run Protocol

ABI QuantStudio 6 Pro/7 Pro:

Appendix 5 – ABI QuantStudio™ 6 Pro/7 Pro; Colour Compensation

Appendix 6 – ABI QuantStudio™ 6 Pro/7 Pro; Run Protocol

Sample Preparation

1. Extract genomic DNA by the method of choice.
2. Refer to section
3. Quality Control and Run Validity on page 10 for information on specimen collection, DNA purification and DNA storage.
4. If the sample has been frozen, ensure that it is fully thawed and vortexed prior to use. Thaw at room temperate (20-25 °C).

Reagent Preparation

1. Take the required number of Olerup QTYPE 11 kit plates from the kit box. Use mixer tubes suitable for molecular biology.
2. Ensure that the master mix is fully thawed and vortexed thoroughly prior to use. Thaw at room temperate (20-25 °C). The master mix is always provided in single-test aliquots.
3. The master mix vials can be briefly spun in a microcentrifuge to ensure that all of the liquid is collected at the bottom of the vial.
4. Do not remove the adhesive aluminium sealing foil from the PCR plate until the test setup is to be performed.

Test Setup

5. Olerup QTYPE 11 tests should be set-up within 30 minutes of thawing the Olerup QTYPE 11 plate and master mix. After set-up the sealed plates can be stored in the dark at +4°C to +8°C for up to 8 hours before commencing PCR. If storing plates, ensure to centrifuge the plate for 2 minutes at a minimum of 1000 rcf after setup and prior to loading the plate on to the real time PCR instrument.
6. Vortex the vial of thawed master mix thoroughly.
7. Each test includes a no template control (NTC). The NTC should be prepared as follows, prior to preparing the reaction mixture:
    i. Transfer 3 µl master mix to a microcentrifuge tube (not provided).
    ii. Add 12 µl molecular biology grade water to the microcentrifuge tube and vortex.
    iii. Set the NTC aside.
8. Prepare the reaction mixture in a disposable reaction tube in accordance with the lot-specific information. With the exception of the NTC, each well of the test should contain 2 µl master mix, 10 ng of DNA and enough molecular biology grade water to give a final reaction volume of 10 µl per well. Each component should be vortexed thoroughly prior to addition to the reaction mixture.
9. Vortex the reaction mixture thoroughly.
10. Transfer the reaction mixture into a disposable reagent reservoir.
11. Using a suitable multi-channel pipette device with disposable filter tips, transfer 10 µl of the reaction mixture into all wells except the NTC well of the test, making sure not to

contaminate the tip with the dried pellet in each well. Refer to lot-specific documentation for information on the wells used in each test.

12. Using a 10 µl pipette with disposable filter tip, transfer 10 µl of the NTC molecular biology grade water-master mix mixture into the NTC well of the test plate.
13. Seal the plate using the provided optically-clear adhesive sealing sheet. Use a PCR Seal Applicator to ensure that the sheet makes full contact with the well chimneys. Care should be taken to ensure that the sealing sheet is kept clean, as any marks (such as fingerprints) can interfere with reading of the fluorescent signal.
14. Centrifuge the plate for 2 minutes at a minimum of 1000 rcf to ensure that no air bubbles are present in the wells. Centrifuge the plate on a support, to make sure no dust or debris from the centrifuge is attached to the outside of the plate, to avoid block contamination.
15. Place the plate in real time PCR Instrument and start the run, using the settings outlined in the Run Protocol sections.
16. Refer to the SCORE 6 instructions for use, section 6 ‘Using SCORE 6’, for details on how to interpret the typing results.

RESULTS/EXPECTED VALUES

SCORE 6 performs the following actions:

The data is assessed for run validity within SCORE 6, i.e., the presence of amplification in the internal positive control in each reaction, and absence in the NTC. The C_q and total fluorescence values for each assay are automatically compared to pre-defined cut-offs held in the SCORE 6 typing kit file, in order to determine whether each assay is positive or negative. The reactivity pattern is compared to the expected specificity for each assay for a given alignment release. The possible alleles that are present in the sample are displayed by SCORE 6 and may be exported or printed as a report.

QUALITY CONTROL AND RUN VALIDITY

Each Olerup QTYPE 11 test includes a negative control well to ensure that there is no DNA contamination of the pipetting instruments, master mix or molecular biology grade water. In the event of amplification in this well, SCORE will display a warning. Refer to SCORE 6 Instructions for use for more information.

WASTE DISPOSAL

The product is classified as conventional waste according to the Commission Regulation (EU) No 1357/2014 on waste. All disposal practices must be in accordance with local, regional, national and international regulations.

Packaging empty containers are treated as conventional waste and sent for recycling or incineration

For further information on safety handling and disposing of Olerup QTYPE 11 products refer to the Safety Data Sheet available on www.caredx.com.

LIMITATIONS OF THE PROCEDURE

1. All laboratory equipment must be calibrated according to the manufacturer's recommendations.
2. The Olerup QTYPE 11 HLA Typing requires highly controlled test conditions to ensure adequate amplification and detections from the target DNA template. The procedure described in this Instructions for Use document must be followed.
3. The extracted genomic DNA sample must have an $OD_{260/280}$ ratio between 1.67 and 2.0. Olerup QTYPE 11 kits have been optimised to use 10ng genomic DNA per well.
4. The reporter dyes used in dually-labelled hydrolysis probes are light sensitive. Olerup QTYPE 11 kit plates are opaque and are provided with an adhesive aluminium foil seal. After this seal has been removed ensure that the PCR is carried out within 30 minutes, if exposed to light and in room temperature. The plate may be stored up to 8 hours if stored dark and in +4°C to +8°C.
5. Make sure that the plate is centrifuged as instructed. If storing plates, ensure to centrifuge the plate for 2 minutes at a minimum of 1000 rcf after setup and prior to loading the plate on the real time PCR instrument.

   It is important to ensure that no air bubbles are present in the reaction mixes during PCR, as they may interfere with the detection of the reporter dye.
6. Olerup QTYPE 11 kit relies on the accurate detection of fluorescent signal in each PCR cycle. Each test is provided with an optically-clear adhesive sealing sheet. No other PCR sealing sheet should be used.

TROUBLESHOOTING

Problem	Reason	Action
Positive Control amplification failure	Wrong DNA concentration	Measure DNA concentration. Olerup QTYPE 11 has been verified with 10-50 ng DNA per reaction well
	The DNA contains PCR inhibitors, e.g. proteins, ethanol (from precipitation steps), remaining matrixes from solid-phase DNA purification products	Measure the DNA quality. Olerup QTYPE 11 has been validated for use with DNA with a A260/A280 ratio of 1.67 – 2.0 by UV spectrophotometry. Follow the supplier's DNA extraction protocol exactly. Re-extract the DNA.
	The DNA has been extracted from heparinized blood.	Use non-heparinized blood or use DNA extraction protocols for heparinized blood.
	The DNA is dissolved in a buffer containing EDTA.	Repeat the DNA extraction and dissolve the DNA in dH_2O.
	Kits are not stored at adequate temperature.	Store the kits in accordance with the package label.
	Bubbles in reaction mixes or inadequately resuspended reaction mixes	Ensure plates are centrifuged for 2 minutes at a minimum of 1000 rcf before PCR.
	Problem with Thermal Cycler	Ensure that the instrument is serviced and the proper calibrations have been done. Ensure that there are no foreign objects in the heat block or on the lenses of the instrument.
	Evaporation	Ensure the PCR sealing sheet is firmly applied. Only use the provided sealing sheets.
	Failure of colour compensation run.	Repeat colour compensation. Ensure that the correct colour compensation plate lot is

		used for the Olerup QTYPE 11 lot.
	Incorrect thermal cycling parameters.	Ensure that the correct thermal cycling parameters are used for the correct instrument type. Ensure the filter and imaging parameters are correct.
Random failure of amplification.	Bubbles in reaction mixes or inadequately resuspended reaction mixes	Ensure plates are centrifuged for 2 minutes at a minimum of 1000 rcf before PCR.
	Problem with Thermal Cycler	Ensure that the instrument is serviced and the proper calibrations have been done. Ensure that there are no foreign objects in the heat block or on the lenses of the instrument.
	Evaporation	Ensure the PCR sealing sheet is firmly applied. Only use the provided sealing sheets.
	Use of non-calibrated pipettes.	Calibrate all pipettes routinely according to the supplier's recommendations.
	Master Mix and sample DNA have not been properly mixed before use.	Mix briefly by vortexing before use. We recommend to vortex after each row.
	Uneven volume of DNA-Master Mix mixture has been added to the wells.	Perform pipetting more carefully.
False positive amplification.	DNA contamination.	Use gloves, pipette tips containing barriers (filter plugs) and separate rooms for pre-PCR handling and post-PCR handling. Assure accurate handling of all samples, in all steps. Check No Template Control for signs of contamination.
	Failure of colour compensation run.	Repeat colour compensation. Ensure that the correct colour

		compensation plate lot is used for the Olerup QTYPE 11 lot.
	Incorrect thermal cycling parameters.	Ensure that the correct thermal cycling parameters are used for the correct instrument type. Ensure the filter and imaging parameters are correct.
False negative amplification.	The thermal cycler is not properly calibrated	Ensure that the instrument is serviced and the proper calibrations have been done. Ensure that there are no foreign objects in the heat block or on the lenses of the instrument.
	Incorrect thermal cycling parameters.	Ensure that the correct thermal cycling parameters are used for the correct instrument type. Ensure the filter and imaging parameters are correct.
	Problem with heat block	Ensure that the heat block is clean and there are no foreign objects in the heat block.

LICENSES & TRADEMARKS

Olerup QTYPE 11 is a registered trademark of CareDx AB.

CleanAmp™ dNTPs for hot start PCR are licensed from Trilink Biotechnologies Inc for use in supplied master mix.

Black Hole Quencher® (BHQ®), CAL Fluor® and Quasar® dye technology incorporated in this product are used under licensing agreement with LGC Biosearch Technologies, Inc. and protected by U.S. and world-wide patents issued or in application.

Roche and LightCycler are trademarks of Roche.

QuantStudio and ViiA are trademarks of Thermo Fisher Scientific and its subsidiaries.

WARRANTY

CareDx AB warrants its products to the original purchaser against defects in materials and workmanship under normal use and application. This warranty applies only to products that have been handled and stored in accordance with CareDx AB's recommendations and does not apply to products that have been the subject of alternation, misuse, or abuse. All claims under this warranty must be directed to CareDx AB in writing and must be accompanied by a copy of the purchaser's invoice. This warranty is in lieu of all other warranties, expressed

or implied, including the warranties of merchantability and fitness for a particular purpose. In no case shall CareDx AB be liable for incidental or consequential damages. This product may not be reformulated, repacked or resold in any form without the written consent of CareDx AB, Franzéngatan 5, SE-112 51 Stockholm, Sweden.

GUARANTEE

CareDx AB guarantees that the primers and probes in the Olerup QTYPE 11 HLA Typing Kits have the specificities given in SCORE 6 Interpretation Software. Note that it is not possible to functionally test all mixes as rare positive DNA samples are unavailable. In these cases, the primers and probes have been tested in combination with other mixes in order to confirm that they have the expected specificity. Refer to the lot-specific information for information on these mixes.

When stored at -15°C to -25°C, the dried primers and probes are stable for 18 months from the date of manufacture. When stored at -15°C to -25°C, the master mix is stable for 18 months from the date of manufacture. Refer to the lot specific kit labels for information on expiry dates.

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3. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantations. Tissue Antigens 1992: 39: 225-235.
4. Holland PM, Abramson RD, Watson R, Gelfand DH. Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of Thermus aquaticus DNA polymerase. PNAS 1991: 88(16): 7276-7280.
5. DNA storage recommendations can be found at www.phe-culturecollections.org.uk/products/dna/howtohandledna.aspx

APPENDIX 1 – LIGHTCYCLER 480 II; COLOUR COMPENSATION

Scope

This is a brief guide to perform Colour compensation run on Roche LightCycler 480 II

Background

In order to reduce fluorescent cross talk between each of the channels, a colour compensation run must be performed before an Olerup QTYPE 11 test can be analysed in SCORE 6. Each individual LightCycler instrument requires its own colour compensation run and each individual Olerup QTYPE 11 lot is compatible to specific colour compensation lot(s). Please refer to the Olerup QTYPE 11 lot specific Product Insert for Olerup QTYPE 11 colour compensation lot compatibility.

On older LightCycler 480 II instruments with a xenon bulb light source (instead of LED light source) the colour compensation run should be repeated each time the bulb is replaced. Refer to the Roche LightCycler 480 II user manual for information on when to replace the xenon bulb.

A new colour compensation run should be performed after instrument maintenance. A new colour compensation run should be performed to ensure lot compatibility between Olerup QTYPE 11 lots and colour compensation lots and every 12 months on both LED and xenon bulb equipped instruments. It is important to ensure lot compatibility as well as that the most recent colour compensation experiment is selected when analysing Olerup QTYPE 11 runs in SCORE 6. For further details, refer to the SCORE 6 instructions for use.

The colour compensation run information is applied at the point of analysis of the Olerup QTYPE 11 test within the SCORE 6. It is important that the correct colour compensation run file is applied during the analysis i.e., the colour compensation file that is applied should have been performed on the same instrument as the Olerup QTYPE 11 test. It is recommended that the colour compensation file is named with the colour compensation lot number, run date and the instrument's serial number or another identifier.

Instrument Settings for Colour Compensation

Before setting up the colour compensation experiment setup the instrument with the following protocols:

Cycles	Stage	Target Temperature (°C)	Hold Time (s)	Ramp Rate (°C/s)
1	Initial Denaturation	95	60	4,8
40	Cycles	98	5	4,8
		65	10	2,5
		72	15*	4,8
1	Colour Compensation	95	1	4,8
		40	30	2,5
		77**	-	-

*fluorescent detection (single acquisition) is performed at the end of this step
**set 'Acquisition' to 'Continuous' with 1 acquisition per °C.

Set the reaction volume to 10 µl.

Set the filter settings to the following, with **Integration Time Mode** set to **Dynamic**:

Name	Excitation Filter (nm)	Emission Filter (nm)	Quant Factor	Max Integration Time (s)
465-510	465	510	10	1
533-580	533	580	10	1
533-610	533	610	10	2
618-660	618	660	10	1

Note: do not change the name of each filter combination.

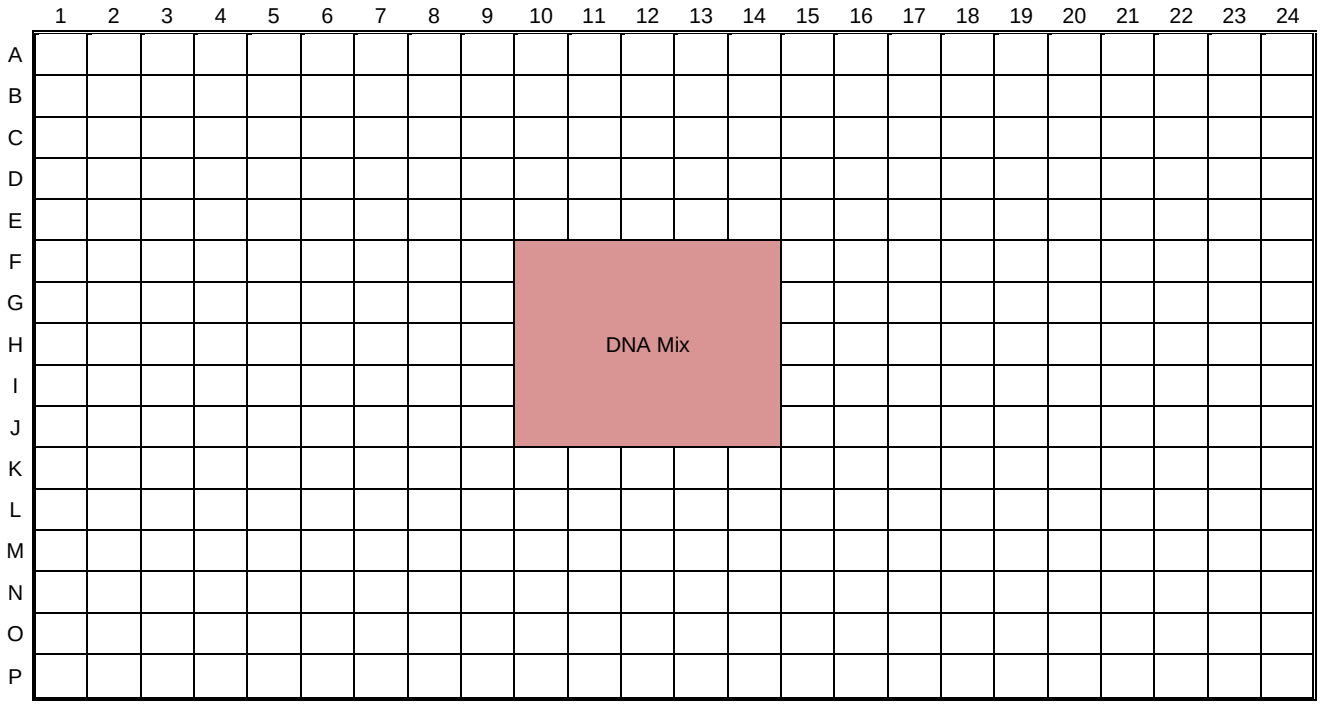
Set the Plate type to ‘White Plates’. This can be done by selecting the tools icon and the instruments setting submenu.

Colour Compensation Setup

The Colour Compensation reagents consist of a 384 well PCR plate with pre-aliquoted and dried reaction mixes, a vial of CC DNA Control and a vial of master mix.

Prepare the reactions as follows:

1. Ensure that the CC DNA Control and master mix are completely defrosted.
2. Vortex thoroughly.
3. Using disposable filter tips add the following to a 1.5 ml microcentrifuge tube, and then briefly vortex:
    a. 60 µl master mix.
    b. 210 µl molecular biology grade water.
    c. 30 µl CC DNA Control.
4. Remove the aluminium foil from the Colour Compensation plate.
5. Using filter tips, dispense 10 µl of the mixture to wells, F10-F14, G10-G14, H10-H14, I10-I14 and J10-J14.



6. Seal the plate using the provided optically-clear adhesive sealing sheet. Ensure that the sheet makes full contact with the well chimneys. Care should be taken to ensure that the sealing sheet is kept clean, as any marks (such as fingerprints) can interfere with reading of the fluorescent signal.
7. Centrifuge the plate for 2 minutes at a minimum of 1000 rcf in order to ensure that no air bubbles are present in the wells. Centrifuge the plate on a support, to make sure no dust or debris from the centrifuge is attached to the outside of the plate, to avoid heat block contamination.
8. Place the plate in the LightCycler 480 II and start the run, using the settings outlined in the Instrument Settings for Colour Compensation section.
9. When the run is complete export the data as a .txt file by clicking on the following icon in the LightCycler software:

The colour compensation should be performed to ensure lot compatibility between Olerup QTYPE 11 lots and colour compensation lots and every 12 months, ideally after instrument service. Include the colour compensation lot number, full date of the colour compensation run and the instrument identifier, such as serial number, in the file name to ensure the correct colour compensation file is used in subsequent analysis.

APPENDIX 2 – LIGHTCYCLER 480 II; RUN PROTOCOL

Scope

This is a brief guide to set up Roche LightCycler 480 II to enable runs of the Olerup QTYPE11 kit.

Run Protocol

Before beginning a run, set up the instrument with the following protocol. Refer to the LightCycler 480 II instructions for use for details on how to set up the instrument.

Cycles	Stage	Target Temperature (°C)	Hold Time (s)	Ramp Rate (°C/s)
1	Initial Denaturation	95	60	4,8
40	Cycles	98	5	4,8
		65	10	2,5
		72	15*	4,8

*fluorescent detection (single acquisition) is performed at the end of this step

Set the reaction volume to 10 µl.

Set the filter settings to the following, with **Integration Time Mode** set to **Dynamic**:

Name	Excitation Filter (nm)	Emission Filter (nm)	Quant Factor	Max Integration Time (s)
465-510	465	510	10	1
533-580	533	580	10	1
533-610	533	610	10	2
618-660	618	660	10	1

Note: do not change the name of each filter combination.

Set the Plate type to ‘White Plates’. This can be done by selecting the tools icon and the instruments setting submenu.

Running the test:

1. Enter at least a sample identifier and Olerup QTYPE 11 kit lot number in the file name. It is recommended that the test date and instrument identifier are also included in the filename.

   When the run is complete export the data as a .txt file by clicking on the following icon in the LightCycler software:

APPENDIX 3 – ABI QUANTSTUDIO™ 6 FLEX/7 FLEX/DX AND VIIA-7; COLOUR COMPENSATION

Scope

This is a brief guide to perform Colour compensation run on ABI QuantStudio 6 Flex/7 Flex/Dx and ViiA-7.

Background

In order to reduce fluorescent cross talk between each of the channels, a colour compensation run must be performed before an Olerup QTYPE 11 test can be analysed in SCORE 6. Each individual ABI instrument requires its own colour compensation run and each individual Olerup QTYPE 11 lot is compatible to specific colour compensation lot(s). Please refer to the Olerup QTYPE 11 lot specific Product Insert for Olerup QTYPE 11 colour compensation lot compatibility.

A new colour compensation run should be performed to ensure lot compatibility between QTYPE 11 lots and colour compensation lots and every 12 months. It is important to ensure lot compatibility as well as that the most recent colour compensation experiment is selected when analyzing Olerup QTYPE 11 runs in SCORE 6. For further details, refer to the SCORE 6 instructions for use.

A new colour compensation run should be performed after instrument maintenance.

The colour compensation run information is applied at the point of analysis of the Olerup QTYPE 11 test within the SCORE 6. It is important that the correct colour compensation run file is applied during the analysis i.e., the colour compensation file that is applied should have been performed on the same instrument as the Olerup QTYPE 11 test. It is recommended that the colour compensation file is named with the colour compensation lot number, run date and the instrument's serial number or another identifier.

Instrument Settings for Colour Compensation

The instrument setup for the Colour Compensation run for the ABI QuantStudio 6 Flex/7 Flex/Dx and ViiA-7 is identical to the run protocol for Olerup QTYPE 11 on the ABI QuantStudio 6 Flex/7 Flex/Dx and ViiA-7. Refer to Appendix 4 – ABI QuantStudio™ 6 Flex/7 flex/Dx and ViiA-7; Run Protocol.

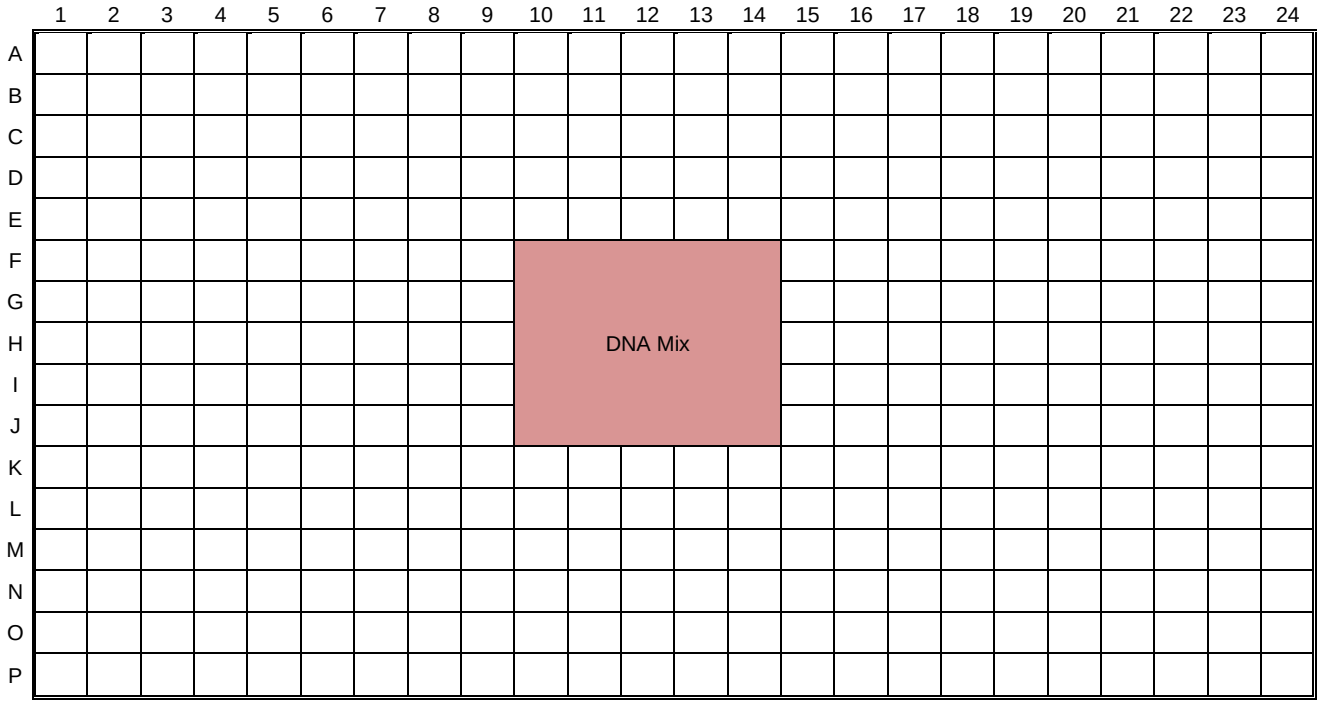
Colour Compensation Setup

The Colour Compensation reagents consist of a 384 well PCR plate with pre-aliquoted and dried reaction mixes, a vial of CC DNA Control and a vial of master mix.

Prepare the reactions as follows:

1. Ensure that the CC DNA Control and master mix are completely defrosted.
2. Vortex thoroughly.
3. Using disposable filter tips add the following to a 1.5 ml microcentrifuge tube, and then briefly vortex:
    a. 60 µl master mix.
    b. 210 µl molecular biology grade water.
    c. 30 µl CC DNA Control.

4. Remove the aluminium foil from the Colour Compensation plate.
5. Using filter tips, dispense 10 µl of the mixture to wells, F10-F14, G10-G14, H10-H14, I10-I14 and J10-J14.



6. Seal the plate using the provided optically-clear adhesive sealing sheet. Ensure that the sheet makes full contact with the well chimneys. Care should be taken to ensure that the sealing sheet is kept clean, as any marks (such as fingerprints) can interfere with reading of the fluorescent signal.
7. Centrifuge the plate for 2 minutes at a minimum of 1000 rcf in order to ensure that no air bubbles are present in the wells. Centrifuge the plate on a support, to make sure no dust or debris from the centrifuge is attached to the outside of the plate, to avoid heat block contamination.
8. Place the plate in the ABI QuantStudio 6 Flex/7 Flex/Dx or ViiA-7and start the run, using the settings outlined in Appendix 4 – ABI QuantStudio™ 6 Flex/7 flex/Dx and ViiA-7; Run Protocol.
9. When the run is complete export the data as a .txt file as outlined in Appendix 4 – ABI QuantStudio™ 6 Flex/7 flex/Dx and ViiA-7; Run Protocol

The colour compensation should be performed to ensure lot compatibility between Olerup QTYPE 11 lots and colour compensation lots and every 12 months, ideally after instrument service. Include the colour compensation lot number, full date of the colour compensation run and the instrument identifier, such as serial number, in the file name to ensure the correct colour compensation file is used in subsequent analysis.

APPENDIX 4 – ABI QUANTSTUDIO™ 6 FLEX/7 FLEX/DX AND VIIA-7; RUN PROTOCOL

Scope

This is a brief guide to set up the ABI QuantStudio 6 Flex/7 Flex/Dx and ViiA-7 to enable runs of the Olerup QTYPE11 kit.

Run Protocol

Before beginning a run, set up the instrument with the following protocol. Refer to the specific instruments' instructions for use for details on how to set up the instrument.

1. On the Experiment Properties Screen make sure the following are selected:
    - The correct instrument type
    - 384 well plate
    - Standard curve
    - Taqman
    - Fast
2. On the Define Screen, the passive dye should be set to 'NONE'
3. On the Run Method screen
    - Change the reaction volume to 10 µl
    - Select the filter combinations X1M1, X2M2, X4M4 and X5M5
    - Program the following cycling parameters

Cycles	Stage	Target Temperature (°C)	Hold Time (s)	Ramp Rate (°C/s)
1	Initial Denaturation	95	60	1,9
40	Cycles	98	5	1,9
		65	10	1,6
		72	16*	1,6

*fluorescent detection is performed at the end of this step

4. On the Export option screen
    - 'Auto Export' should be active
    - 'Format' should be set to ViiA7, ViiA7 v1.2.x, ViiA7 v1.2.x fixed, QuantStudio 6 Flex&7 Flex or QuantStudio Dx/ ViiA7
    - 'Export Data To' should be set to One File
    - Click on 'Browse' to select the default export file location
    - 'File Type' should be set to .txt
    - Of the various tabs, only 'Raw Data' should be selected

Running the test:

1. Enter at least a sample identifier and Olerup QTYPE 11 kit lot number in the file name. It is recommended that the test date and instrument identifier are also included in the filename.
2. When the run is complete export the data as a .txt file according with the options selected as described above.

APPENDIX 5 – ABI QUANTSTUDIO™ 6 PRO/7 PRO; COLOUR COMPENSATION

Scope

This is a brief guide to perform colour compensation run on the ABI QuantStudio 6 Pro/7 Pro.

Background

In order to reduce fluorescent cross talk between each of the channels, a colour compensation run must be performed before an Olerup QTYPE 11 test can be analysed in SCORE 6. Each individual ABI instrument requires its own colour compensation run and each individual Olerup QTYPE 11 lot is compatible to specific colour compensation lot(s). Please refer to the Olerup QTYPE 11 lot specific Product Insert for Olerup QTYPE 11 colour compensation lot compatibility.

A new colour compensation run should be performed to ensure lot compatibility between Olerup QTYPE 11 lots and colour compensation lots and every 12 months. It is important to ensure lot compatibility as well as that the most recent colour compensation experiment is selected when analysing Olerup QTYPE 11 runs in SCORE 6. For further details, refer to the SCORE 6 Instructions for Use.

A new colour compensation run should be performed after instrument maintenance.

The colour compensation run information is applied at the point of analysis of the Olerup QTYPE 11 test within the SCORE 6. It is important that the correct colour compensation run file is applied during the analysis i.e. the colour compensation file that is applied should have been performed on the same instrument as the Olerup QTYPE 11 test. It is recommended that the colour compensation file is named with the colour compensation lot number, run date and the instrument's serial number or another identifier.

Instrument Settings for Colour Compensation

The instrument setup for the Colour Compensation run for the ABI QuantStudio 6 Pro/7 Pro is identical to the run protocol for Olerup QTYPE 11 on the ABI QuantStudio 6 Pro/7 Pro. Refer to Appendix 6 – ABI QuantStudio™ 6 Pro/7 Pro; Run Protocol.

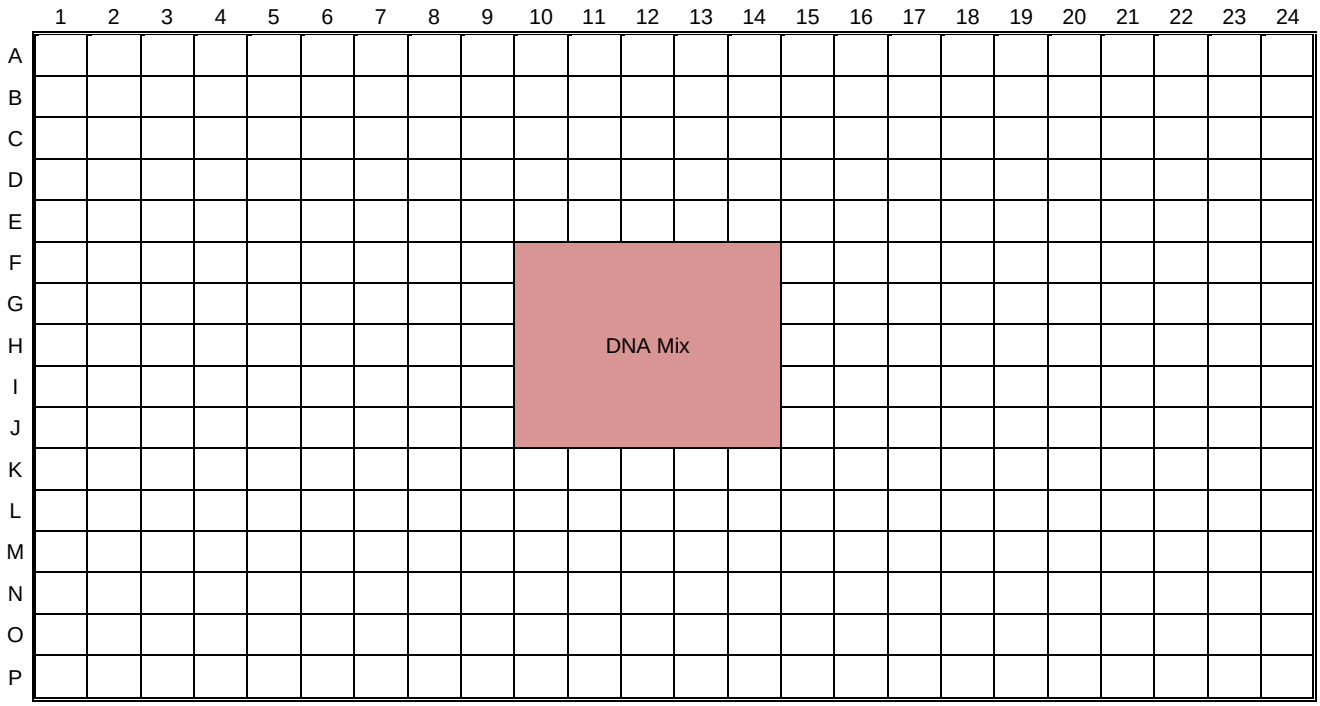
Colour Compensation Setup

The Colour Compensation reagents consist of a 384 well PCR plate with pre-aliquoted and dried reaction mixes, a vial of CC DNA Control and a vial of master mix.

Prepare the reactions as follows:

1. Ensure that the CC DNA Control and master mix are completely defrosted.
2. Vortex thoroughly.
3. Using disposable filter tips add the following to a 1.5 ml microcentrifuge tube, and then briefly vortex:
    a. 60 µl master mix.
    b. 210 µl molecular biology grade water.
    c. 30 µl CC DNA Control.

4. Remove the aluminium foil from the Colour Compensation plate.
5. Using filter tips, dispense 10 µl of the mixture to wells, F10-F14, G10-G14, H10-H14, I10-I14 and J10-J14.



6. Seal the plate using the provided optically-clear adhesive sealing sheet. Ensure that the sheet makes full contact with the well chimneys. Care should be taken to ensure that the sealing sheet is kept clean, as any marks (such as fingerprints) can interfere with reading of the fluorescent signal.
7. Centrifuge the plate for 2 minutes at a minimum of 1000 rcf in order to ensure that no air bubbles are present in the wells. Centrifuge the plate on a support, to make sure no dust or debris from the centrifuge is attached to the outside of the plate, to avoid heat block contamination.
8. Place the plate in the ABI QuantStudio 6 Pro/7 Pro and start the run, using the settings outlined in Appendix 6 – ABI QuantStudio™ 6 Pro/7 Pro; Run Protocol.
9. When the run is complete, export the data as a .txt file as outlined in Appendix 6 – ABI QuantStudio™ 6 Pro/7 Pro; Run Protocol.

The colour compensation should be performed to ensure lot compatibility between Olerup QTYPE 11 lots and colour compensation lots and every 12 months, ideally after instrument service. Include the colour compensation lot number, full date of the colour compensation run and the instrument identifier, such as serial number, in the file name to ensure the correct colour compensation file is used in subsequent analysis.

APPENDIX 6 – ABI QUANTSTUDIO™ 6 PRO/7 PRO; RUN PROTOCOL

Scope

This is a brief guide to set up the ABI QuantStudio 6 Pro/7 Pro to enable runs of the Olerup QTYPE 11 kit.

Run Protocol

Before beginning a run, set up the instrument with the following protocol. Refer to the specific instruments' instructions for use for details on how to set up the instrument.

1. On the System Template Screen make sure the following are selected:
    - The correct instrument type
    - 384-well block
    - Standard curve analysis
    - Fast run mode
2. On the Run Method screen
    - Change the reaction volume to 10µl
    - In 'Filter Settings', select filter combinations X1M1, X2M2, X4M4 and X5M5
    - Program the following cycling parameters:

Cycles	Stage	Target Temperature (°C)	Hold Time (s)	Ramp Rate (°C/s)
1	Initial Denaturation	95	60	1,9
40	Cycles	98	5	1,9
		65	10	1,6
		72	16*	1,6

*Fluorescent detection is performed at the end of this step

3. On the Plate Setup screen, the 'Passive Reference' should be set to 'NONE'.
4. Run the test according to the instructions below.
5. On the Export Settings screen, open a default export setting and make the following changes:
    - 'Analysis Module' should be set to 'Primary'
    - In 'View', make sure only 'Raw Data' is selected
    - In 'Options' make sure only 'Section header' is selected
    - Save this export setting as 'Score 6'
6. To export, go to the 'Quality Check' tab
    - Click 'Actions' and select 'Export'
    - Set the file format to .txt
    - In 'Export Settings', select 'Score 6'

Running the test:

1. Enter at least a sample identifier and QTYPE kit lot number in the file name. It is recommended that the test date and instrument identifier are also included in the filename.
2. When the run is complete, export the data as a .txt file according to step 6. of the Run Protocol instructions above.

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Changes in revision 0175-LBL v10 compared to 0175-LBL v09.

1. Adding symbol for manufacturer