PlexPCR® Colour Compensation

Colour Compensation kit for *PlexPCR*® kits run on LightCycler® 480 and cobas® z 480 analyzer

CE IVD

Product	Platform	Size (reactions)	Catalogue no.
PlexPCR® Colour Compensation	LC480 I LC480 II z 480	2	REF **90001**

EC REP

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FOR PROFESSIONAL USE ONLY*

Not for sale in the USA

Contents

1 Product description

The LightCycler® 480 Instrument I and II (LC480 I and LC480 II) and the cobas® z 480 analyzer (z 480) instruments are able to simultaneously detect and analyse more than one fluorescent dye in each reaction in real-time PCR (qPCR). This allows more than one target to be identified in multiplex. However, due to the overlap in the emission spectra of the dyes, each dye may be detected in more than one channel, in a phenomenon called 'crosstalk'. To correct for the crosstalk, a Colour Compensation (CC) file is created on the instrument that contains information on each dye spectra and must be applied before analysis of multiplex qPCR reactions.

The ***Plex****PCR*® Colour Compensation kit is for the generation of a ***Plex****PCR*® CC file on the LC480 I, LC480 II and z 480 instruments. All ***Plex****PCR*® kits and ***Resistance****Plus*® kits use a specific combination of dyes, and the ***Plex****PCR*® CC file **MUST** be applied for correct analysis of results.

2 Intended use

This supplementary protocol is intended **ONLY** for users of the LC480 I, LC480 II and z 480 Instruments using ***Plex****PCR*® kits or ***Resistance****Plus*® kits.

CC files can be generated before, during or after performing the ***Plex****PCR*® kits, and the CC file can be saved for later use.

The CC file is specific for the instrument it is created on, therefore must be generated for each instrument prior to analysis.

3 Kit contents

96-well plate: Sufficient for 2 colour compensation files for 20 µl reaction volume. See **Section 8** for setup details.

384-well plate: Sufficient for 5 colour compensation files for 10 µl reaction volume. See **Section 12** for setup details.

Table 1. Kit contents

Cap colour	Contents	Description	Quantity
Blue	***Plex*** Mastermix, 2x	Mastermix containing components necessary for qPCR including dNTPs, $MgCl_2$, DNA polymerase and buffer	1 x 1 ml
Brown	488 CC Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of CC target in 488 nm emission channel on LC480 II (see **Table 10** for emission channel on LC480 I and z 480)	1 x 15 µl
Green	510 CC Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of CC target in 510 nm emission channel on LC480 II (see **Table 10** for emission channel on LC480 I and z 480)	1 x 15 µl
Yellow	580 CC Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of CC target in 580 nm emission channel on LC480 II (see **Table 10** for emission channel on LC480 I and z 480)	1 x 15 µl
Purple	610 CC Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of CC target in 610 nm emission channel on LC480 II (see **Table 10** for emission channel on LC480 I and z 480)	1 x 15 µl
White	640 CC Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of CC target in 640 nm emission channel on LC480 II (see **Table 10** for emission channel on LC480 I and z 480)	1 x 15 µl
Black	660 CC Mix, 20x	Mix containing oligonucleotides^ for amplification and detection of CC target in 660 nm emission channel on LC480 II (see **Table 10** for emission channel on LC480 I and z 480)	1 x 15 µl
Neutral	CC Template#	Template for colour compensation reaction	1 x 400 µl
Light blue	Nuclease Free Water	PCR grade water	1 x 1 ml

Store template tubes separately from oligo mixes, i.e. template or nucleic acid handling room

^ Oligonucleotides are PCR primer pairs, ***Plex****Zyme*® enzymes and fluorescent probe

4 Shipping and storage

- The components of the ***Plex****PCR*® CC kit are shipped on dry ice or ice gel packs. All components should be stored at -20°C upon receipt.
- When stored under the recommended conditions and handled correctly, activity of the kit is retained until the expiry date stated on the label. Do not use past expiry date.

5 General precautions

- PCR tests are prone to contamination from previous PCR products. Never open reaction vessels after the completion of PCR.
- Basic precautions for preventing contamination of PCR reactions include the use of sterile filter pipette tips, use of a new pipette tip for every pipetting action and separation of workflow.
- It is recommended to perform sample preparation/extraction, mastermix preparation, sample addition and thermocycling in spatially separated spaces. At a minimum the PCR instrument should be located in a separate room to areas where reactions are prepared.
- It is recommended to follow routine laboratory precautions. Wear appropriate personal protective equipment such as gloves, protective eye wear and laboratory coat when handling reagents.
- Refer to the product SDS for further safety information.

6 Materials required but not provided

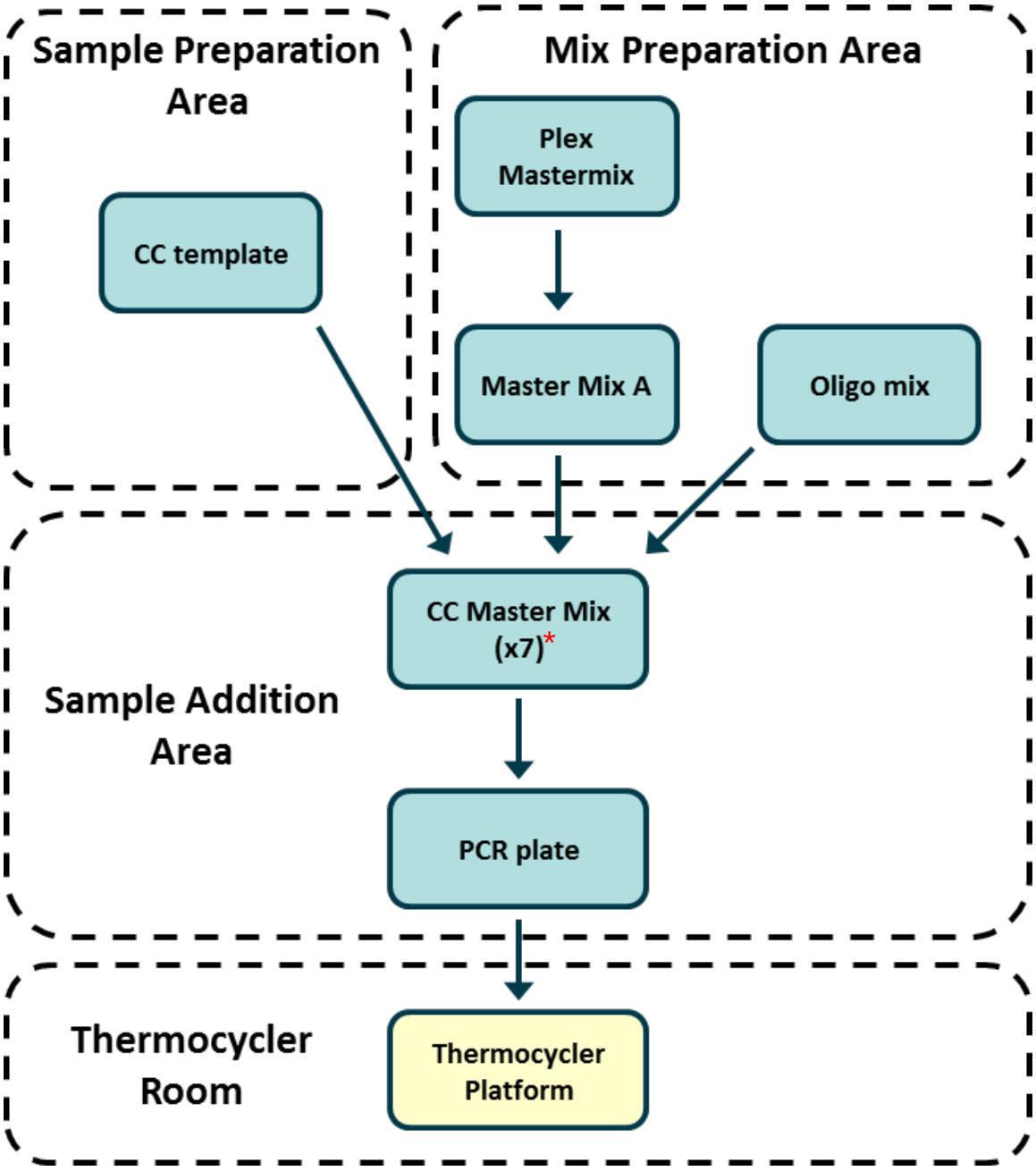
General lab consumables

- Gloves and clean lab coats
- Vortex mixer
- Benchtop centrifuge for 0.5 ml and 1.5 ml tubes
- Micropipettors
- Sterile aerosol-resistant pipette tips
- 0.5 ml tubes and 1.5 ml tubes (PCR-grade)

Instruments and instrument components

- LightCycler® 480 Instrument I
- LightCycler® 480 Instrument II
- cobas® z 480 analyzer
- LightCycler® 480 Thermal Block Cycler Unit, 96-well (Roche, Cat no. 05015219001)
- LightCycler® 480 Thermal Block Cycler Unit, 384-well (Roche, Cat no. 05015197001)
- LightCycler® 480 Multiwell Plate 96 (Roche, Cat no 04729692001)
- LightCycler® 480 Multiwell Plate 384 (Roche, Cat no 04729749001)
- LightCycler® 480 Sealing Foil (Roche, Cat no 04729757001)

7 Procedure overview



* For z 480, the 488 CC Mix is omitted, therefore only 6 CC Master Mixes are required (**Tables 2, 3, 5-9, 15, 16 and 18-22**)

8 Colour Compensation

The ***Plex****PCR*® Colour Compensation kit can be performed at a final reaction volume of 20 µL on a 96 well plate, or at a final reaction volume of 10 µL on a 384 well plate. Refer to **Section 12** for instructions for performing 10 µL reactions on a 384 well plate.

8.1 Preparation of Master Mixes for 20 µl reaction volume on a 96 well plate

Note: Before use of reagents, thaw completely, and mix thoroughly by briefly vortexing.

To create the CC file, 3 replicate reactions of each reporter dye (6 dyes) are required. In addition, 3 replicate control reactions with no dye, are also run. Therefore a total of 7 mixes (6 dyes and 1 with no dye), each with 3 replicates are required.

The following outlines a protocol to make CC Master Mixes (7 mixes) which contains components required for qPCR as well as addition of CC templates. The mixes for the 3 replicate reactions are made in excess (extra 2 reactions for total of 5 reactions).

More specifically, each reaction has a 20 µl final volume, composed of 14 µl Master Mix A (**Table 2**), 5 µl CC template, and 1 µl each reporter dye CC Mix (**Table 4-Table 9**), or 1 µl of water (**Table 3**). This is directly plated into the PCR plate and run on the qPCR instrument.

Seal plate, centrifuge the plate and transfer to thermocycler.

Note: The 488 CC Mix is not required for z 480, therefore **Table 4** can be omitted

Note: Do NOT store excess mixes

Table 2. Master Mix A for 20 µl reaction volume

Reagent	Concentration	Volume (for 37 reactions)
Plex Mastermix (**BLUE**)	2x	370 µl (37 x 10 µl)
Nuclease Free Water (**LIGHT BLUE**)	N/A	148 µl (37 x 4 µl)
Total volume (µl)		518 µl (37 x 14 µl)

Table 3. CC Master Mix (BLANK)

Reagent	Concentration	Volume (5 reactions)
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
Nuclease Free Water (**LIGHT BLUE**)	N/A	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

Table 4. 488 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
488 CC Mix (**BROWN**)	20x	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

Table 5. 510 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
510 CC Mix (**GREEN**)	20x	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

Table 6. 580 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
580 CC Mix (**YELLOW**)	20x	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

Table 7. 610 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
610 CC Mix (**PURPLE**)	20x	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

Table 8. 640 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
640 CC Mix (**WHITE**)	20x	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

Table 9. 660 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 2**)	N/A	70 µl (5 x 14 µl)
660 CC Mix (**BLACK**)	20x	5 µl (5 x 1 µl)
CC template (**NEUTRAL**)	N/A	25 µl (5 x 5 µl)
Total volume (µl)		100 µl (5 x 20 µl)
Load 20 µl onto PCR plate		

8.2 Programming and analysis

Details for programming and analysis are described in **Section 9** and **Section 11**.

8.3 Applying the PlexPCR® CC file

The ***Plex****PCR*® CC file can be subsequently applied to all ***Plex****PCR*® kits or ***Resistance****Plus*® kits run files generated on the same instrument at the same reaction volume. Please refer to the ***Plex****PCR*® CC kit IFU or Product specific IFU for the details on how to apply the ***Plex****PCR*® CC file.

9 Appendix 1a: Programming the LightCycler® 480 and z 480 for 20 µL reaction volume

The following information is based on LightCycler 480 Software (version 1.5).

Detection Format

Create a custom **Detection Format**

Open Tools > **Detection Formats**

Create a New Detection Format, and name 'SpeeDx PlexPCR' (See **Figure 1-Figure 3**)

For **Filter Combination Selection** select the following (Excitation-Emission):

Table 10. Filter combinations^

	488 mix	510 mix	580 mix	610 mix	640 mix	660 mix
LC480 I	450-500	483-533	523-568	558-610	558-640	615-670
LC480 II	440-488	465-510	533-580	533-610	533-640	618-660
z 480	n/a	465-510	540-580	540-610	540-645	610-670

^ These Filter Combinations are the default names for the channels

Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Name Filter Combinations as in **Table 10** (default names)

Figure 1. Custom SpeeDx Detection Format – LC480 II

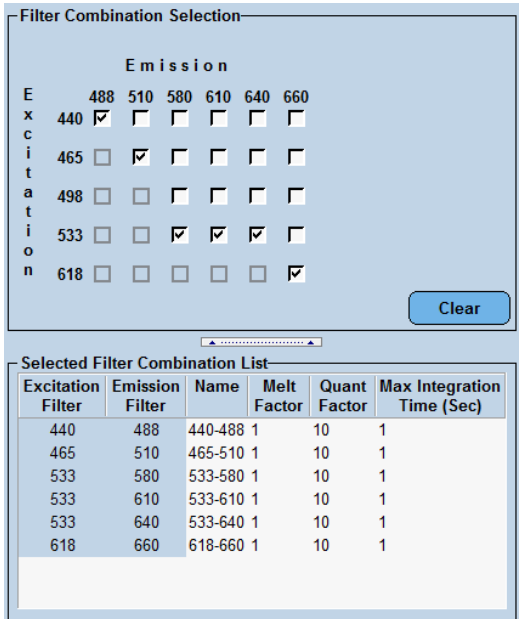


Figure 2. Custom SpeeDx Detection Format – LC480 I

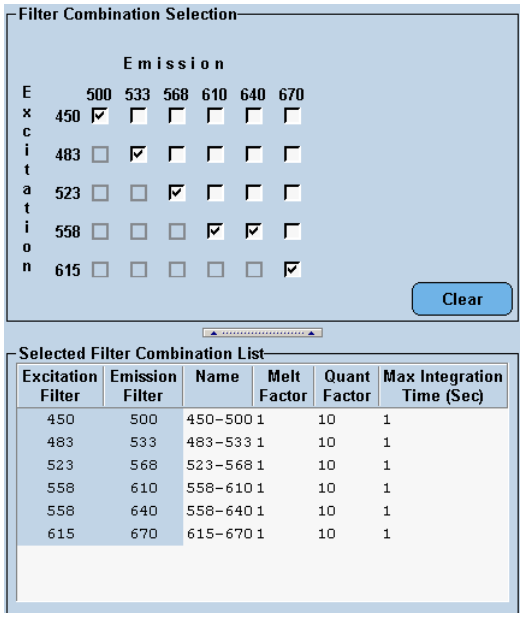
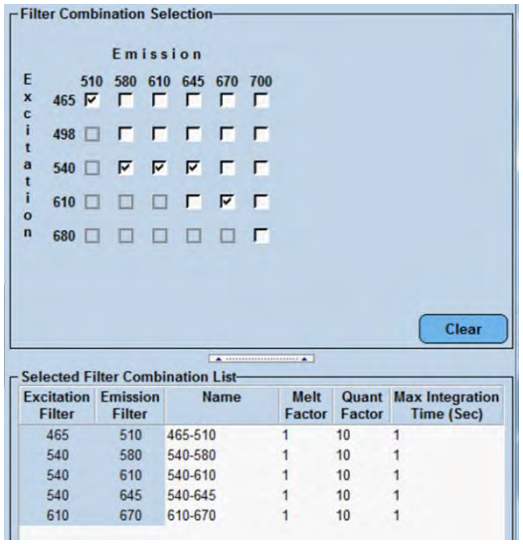


Figure 3. Custom SpeeDx Detection Format – z 480



Instrument Settings

Create a custom **Detection Format**

Open Tools > **Instruments**

For **Instrument Settings** > select **Barcode Enabled**

Experiment setup

Select **New Experiment**

In the **Run Protocol** tab

For **Detection Format** select the custom 'SpeeDx PlexPCR'

Select **Customize** > Select **Integration Time Mode** > **Dynamic**

Set **Reaction Volume** > 20 µl

Create the following Program (shown in more detail in **Figure 4-Figure 8**):

Table 11. Thermocycling program

Program name	Cycles	Target °C	Hold	Ramp rate (°C/s)≠
Polymerase activation	1	95°C	2 min	4.4
Touch down cycling$^{\delta}$: Step down -0.5°C/cycle	10	95°C	5 s	4.4
		61°C – 56.5°C$^{\delta}$	30 s	2.2
Quantification cycling^{+}: Acquisition/Detection	40	95°C	5 s	4.4
		52°C^{+}	40 s	2.2
Colour compensation$^{\gamma}$	1	95°C	10 s	4.4
		45°C	30 s	2.2
		70°C$^{\gamma}$	--	Default
Cooling	1	40°C	30 s	2.2

≠ *Default ramp rate (96 well plate)*

$^{\delta}$ ***Step size:*** *-0.5°C/Cycle,* ***Sec Target:*** *56°C*

$^{+}$ ***Analysis mode:*** *Quantification,* ***Acquisition mode****: Single*

$^{\gamma}$ ***Analysis mode:*** *Colour Compensation,* ***Acquisition mode:*** *Continuous,* ***Acquisitions (per °C):*** *5*

> **Start Run**

Figure 4. Thermocycling program – Polymerase activation

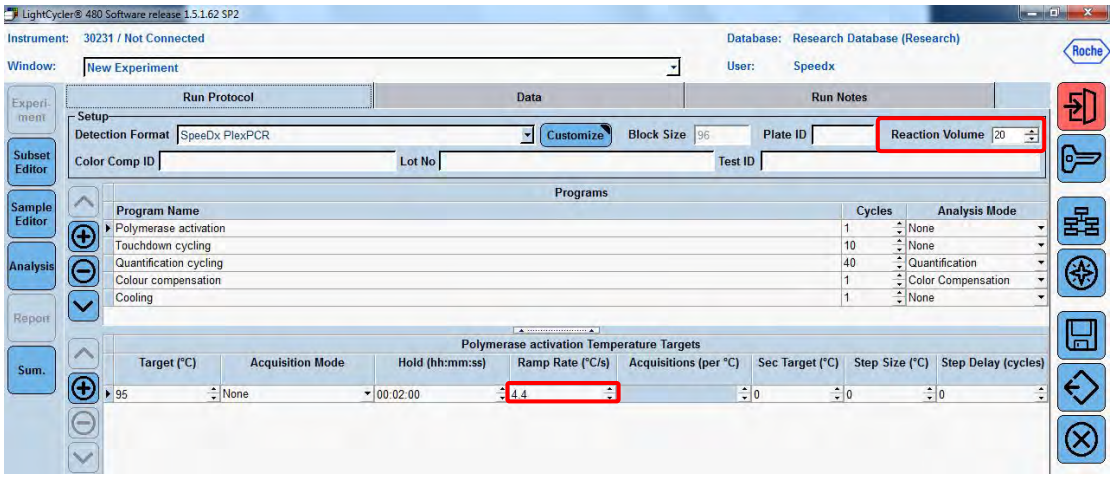


Figure 5. Thermocycling program – Touchdown cycling

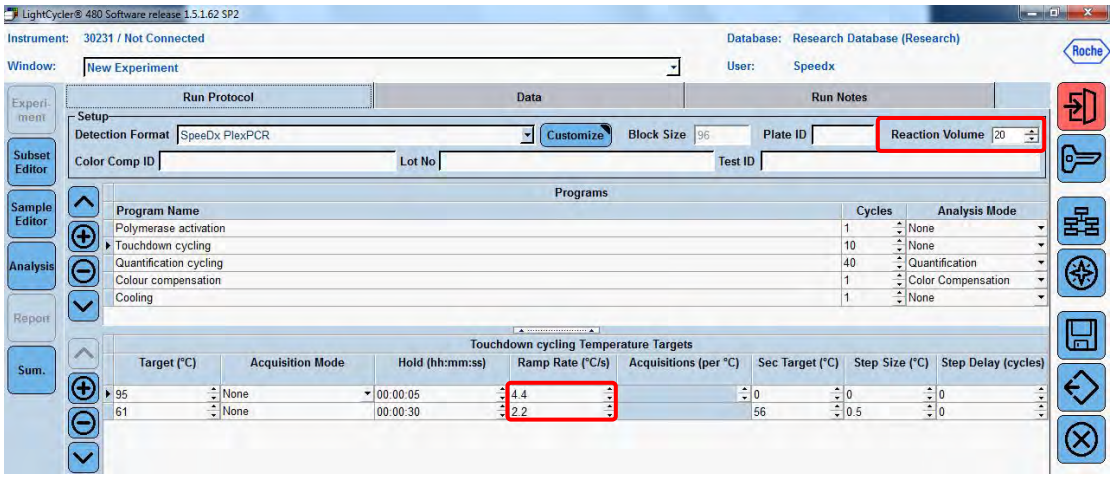


Figure 6. Thermocycling program – Quantification cycling

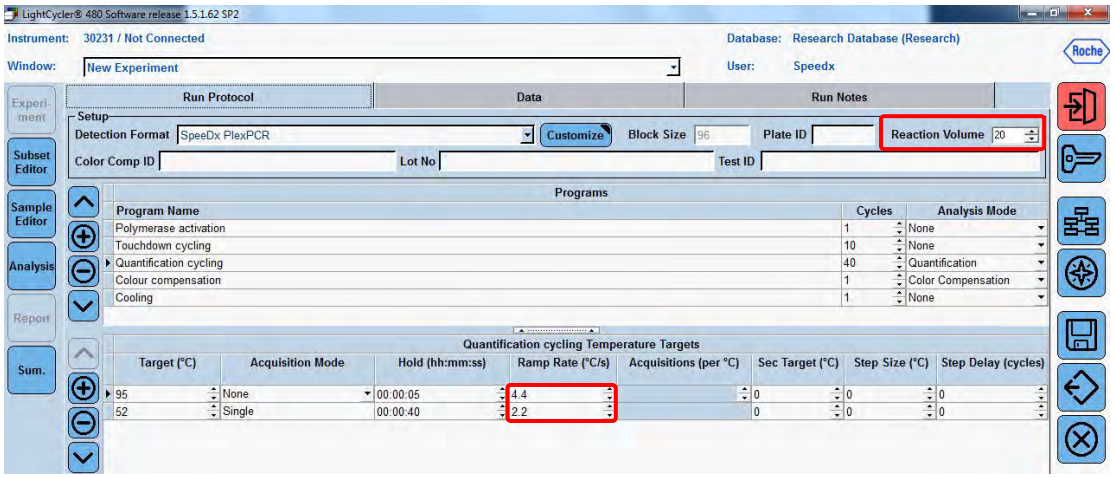


Figure 7. Thermocycling program – Colour compensation

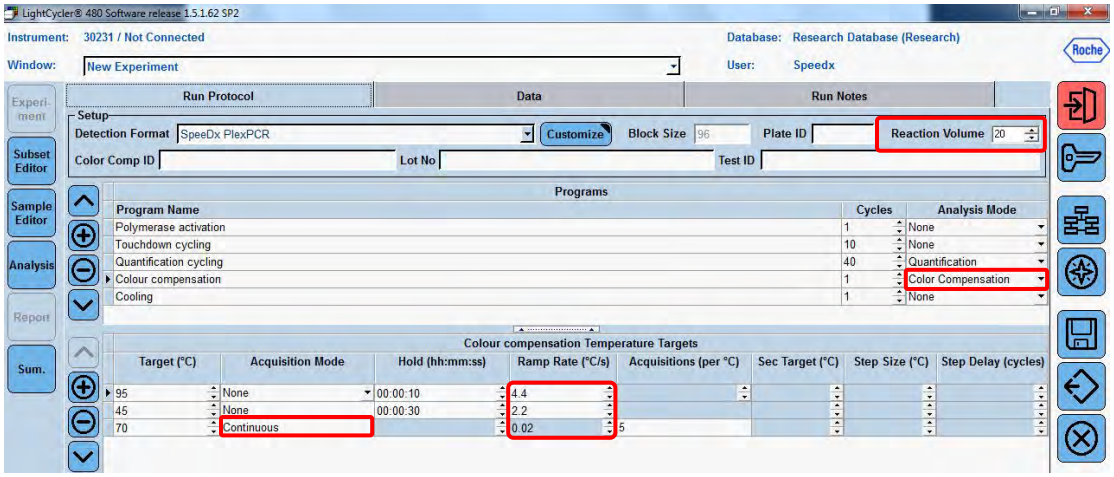
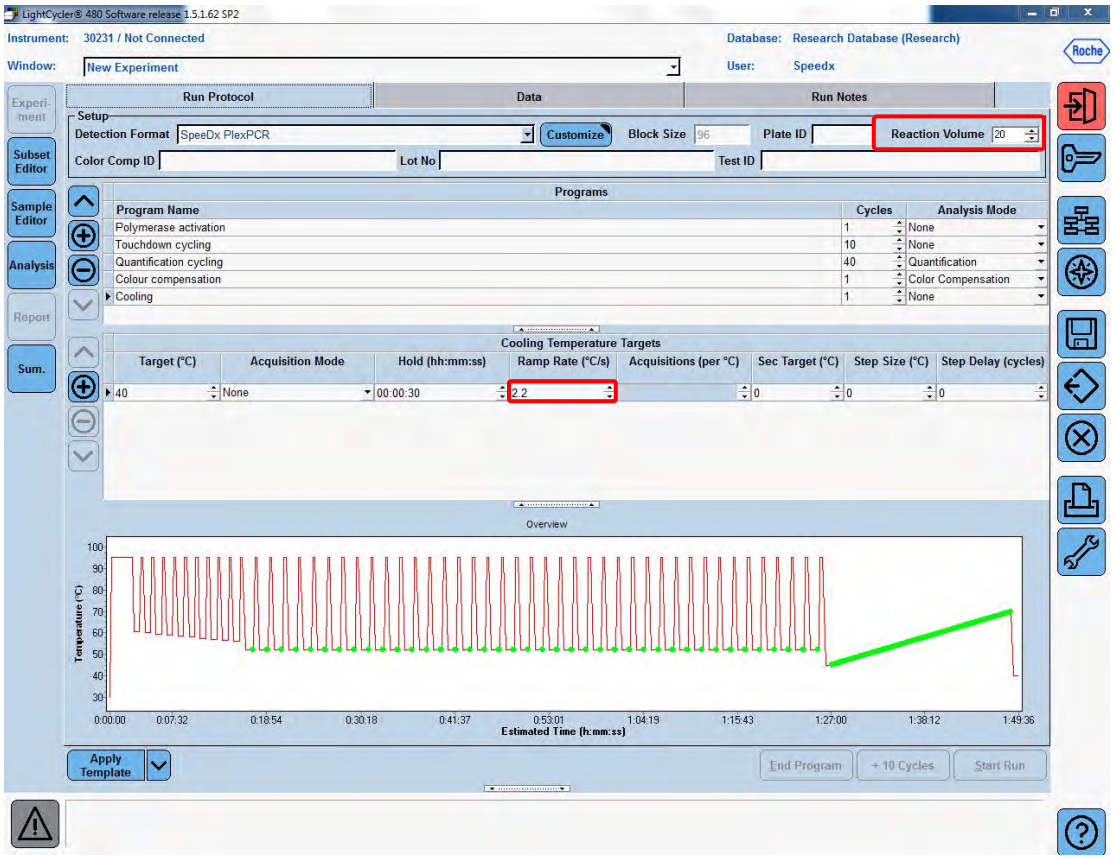


Figure 8. Thermocycling program – Cooling



10 Appendix 1b: Programming the LightCycler® 480 for 10 µL reaction volume

The following information is based on LightCycler 480 Software (version 1.5).

Detection Format

Create a custom **Detection Format**

Open Tools > **Detection Formats**

Create a New Detection Format, and name 'SpeeDx PlexPCR' (See **Figure 9** and **Figure 10**)

For **Filter Combination** Selection select the following (Excitation-Emission):

Table 12. Filter combinations^

	488 mix	510 mix	580 mix	610 mix	640 mix	660 mix
LC480 I	450-500	483-533	523-568	558-610	558-640	615-670
LC480 II	440-488	465-510	533-580	533-610	533-640	618-660

^ These Filter Combinations are the default names for the channels

Set the **Selected Filter Combination List** for all channels as:

Melt Factor: 1

Quant Factor: 10

Max Integration Time (sec): 1

Name Filter Combinations as in **Table 12** (default names)

Figure 9. Custom SpeeDx Detection Format – LC 480 II

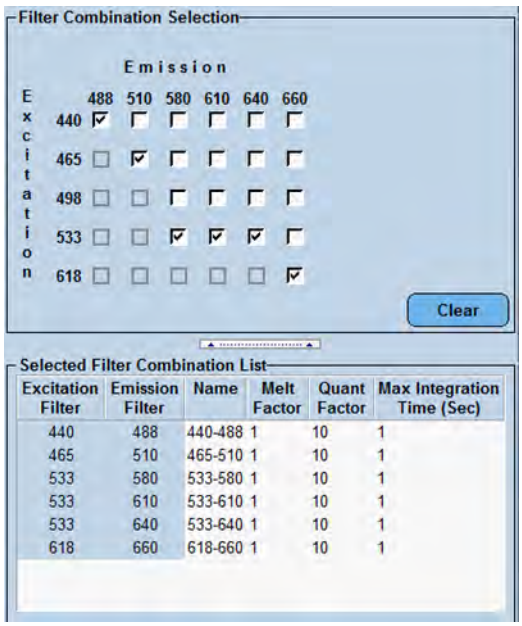
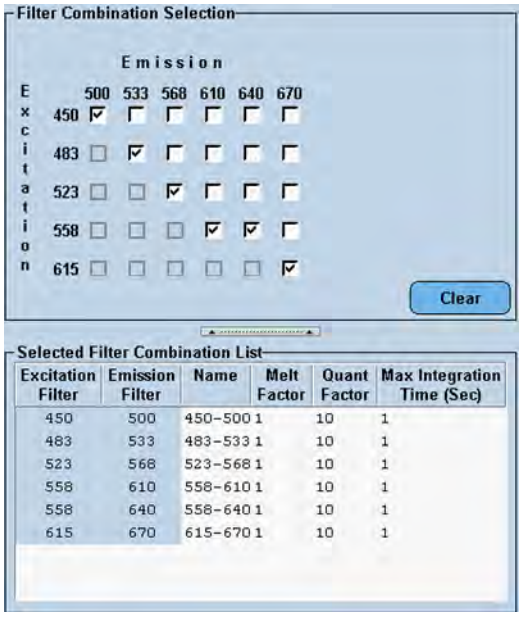


Figure 10. Custom SpeeDx Detection Format – LC 480 I



Instrument Settings

Create a custom **Detection Format**

Open Tools > **Instruments**

For **Instrument Settings** > select **Barcode Enabled**

Experiment setup

Select **New Experiment**

In the **Run Protocol** tab

For **Detection Format** select the custom 'SpeeDx PlexPCR'

Select **Customize** > Select **Integration Time Mode** > **Dynamic**

Set **Reaction Volume** > 10 µl

Create the following Program (shown in more detail in **Figure 11-Figure 15**):

Table 13. Thermocycling program

Program name	Cycles	Target °C	Hold	Ramp rate (°C/s)≠
Polymerase activation	1	95°C	2 min	4.8
Touch down cycling δ: Step down -0.5°C/cycle	10	95°C	5 s	4.8
		61°C – 56.5°C δ	30 s	2.5
Quantification cycling +: Acquisition/Detection	40	95°C	5 s	4.8
		52°C +	40 s	2.5
Colour compensation ¥	1	95°C	10 s	4.8
		45°C	30 s	2.5
		70°C ¥	--	Default
Cooling	1	40°C	30 s	2.5

≠ *Default ramp rate (384 well plate)*

δ ***Step size:*** *-0.5°C/Cycle,* ***Sec Target:*** *56°C*

\+ ***Analysis mode:*** *Quantification,* ***Acquisition mode****: Single*

¥ ***Analysis mode:*** *Colour Compensation,* ***Acquisition mode:*** *Continuous,* ***Acquisitions (per °C):*** *5*

> **Start Run**

Figure 11. Thermocycling program – Polymerase activation

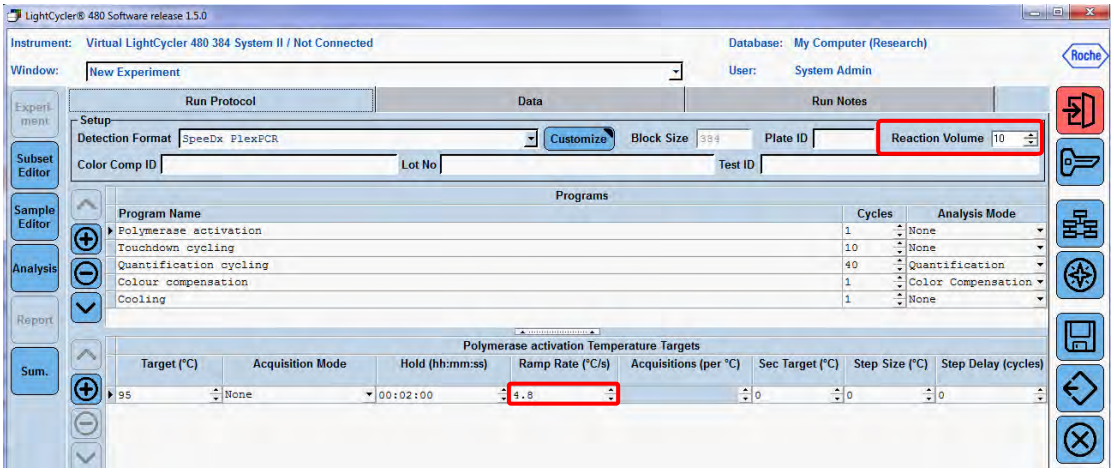


Figure 12. Thermocycling program – Touchdown cycling

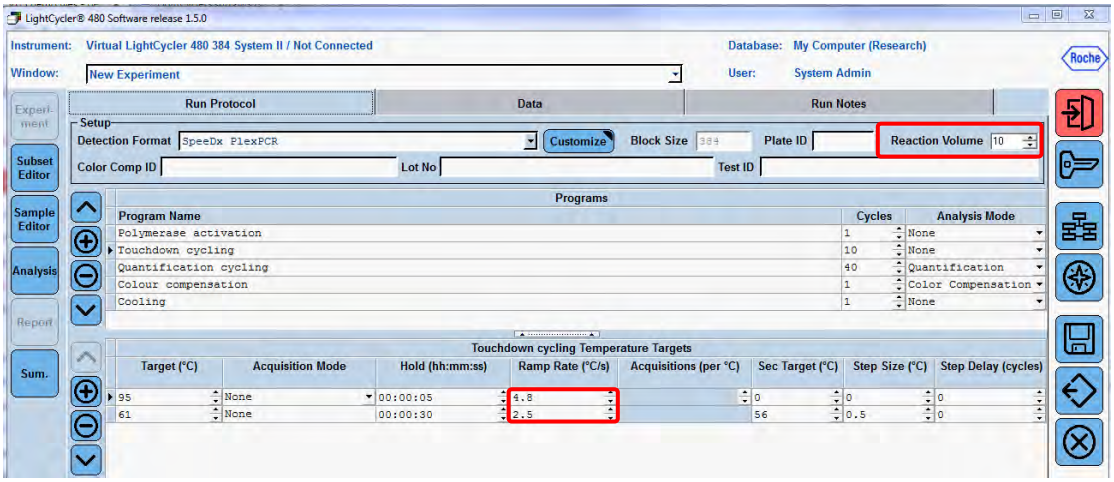


Figure 13. Thermocycling program – Quantification cycling

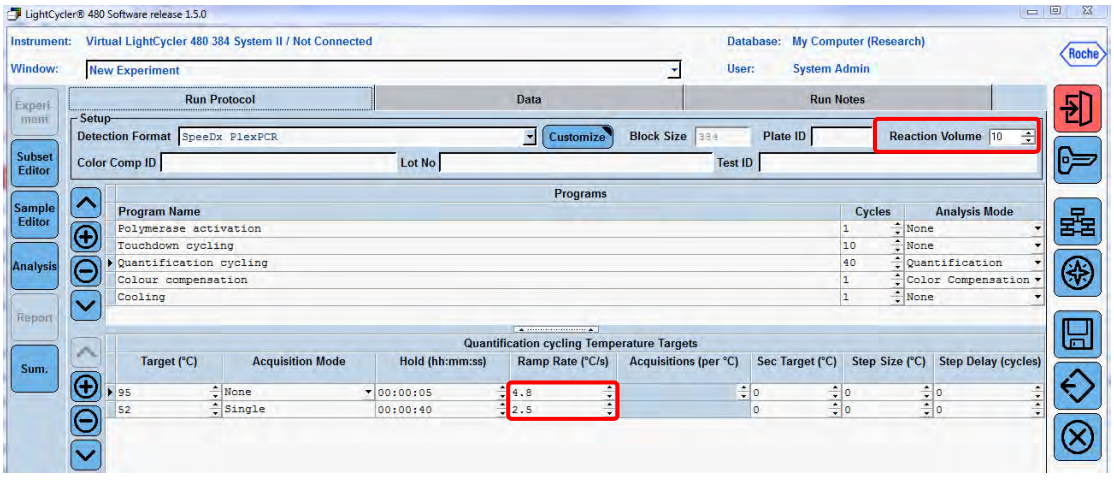


Figure 14. Thermocycling program – Colour compensation

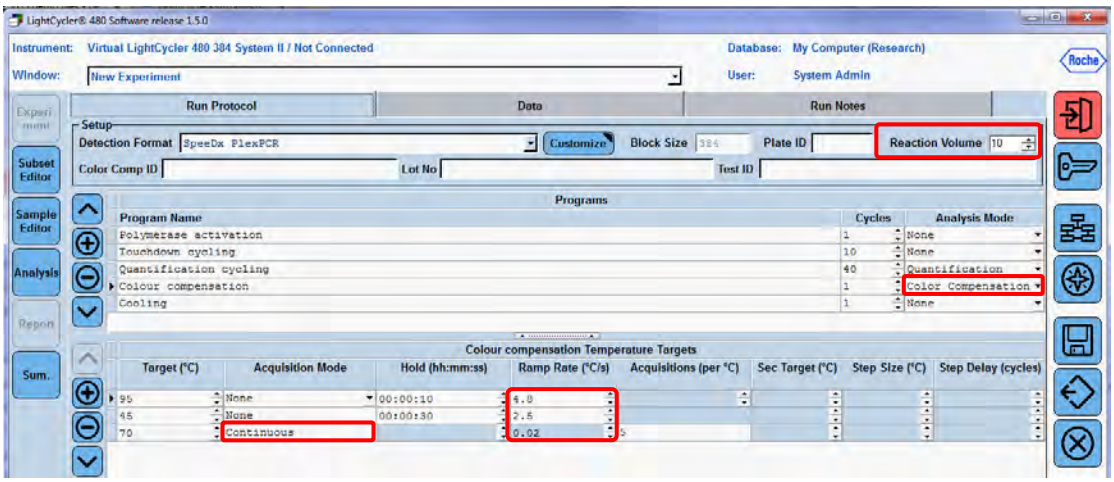
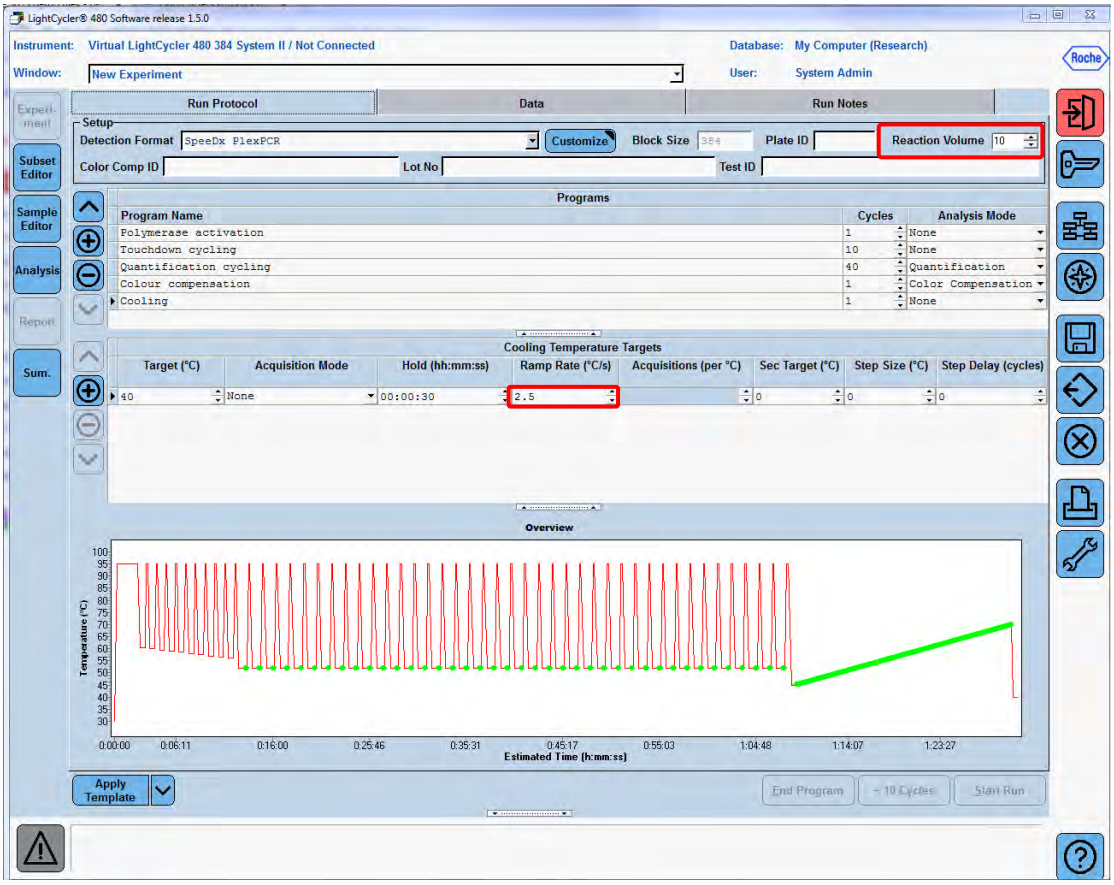


Figure 15. Thermocycling program – Cooling



11 Appendix A: Analysis of CC experiment

The following information is based on LightCycler 480 Software (version 1.5).

The PlexPCR® Colour Compensation kit must be run and applied before analysis of PlexPCR® kits or ResistancePlus kits. This kit can be supplied on request. Please contact sales@speedx.com.au for more information.

Select **Subset Editor** to create a subset

> **Subsets** > Rename the subset "**SpeeDx PlexPCR**"

> Once renamed, in **SpeeDx PlexPCR Settings**

> Select all colour compensation reactions (6 dyes and 1 blank, 3 replicates for each reaction – 21 wells total)

> Select **Apply** (to save any subsequent changes, again select Apply)

Assign replicates for CC Mixes

Select **Sample Editor** >

> **Step 1: Select Workflow** > Select **Color Comp**

> **Step 2: Select Samples** > Select the subset created from above "**SpeeDx PlexPCR**"

> Select replicate wells to assign the following properties

> **Step 3: Edit Color Comp Properties** and select the correct dominant channel and name each reaction as shown in **Table 14** below

Table 14. Sample Name for colour compensation reactions

	Reactions						
Mix Name	**CC Master mix (BLANK)**	**488 mix**	**510 mix**	**580 mix**	**610 mix**	**640 mix**	**660 mix**
Dominant Channel*	Water	440-488	465-510	533-580	533-610	533-640	618-660
Sample Name	BLANK	440-488	465-510	533-580	533-610	533-640	618-660

* Filter Combinations for LC480 II, for LC480 I and z 480 see **Table 10**

Select **Analysis** > **Create New Analysis** > **Color Compensation**

> Colour samples by selecting **Color compensation sample types**

> **Calculate**

*To check the colour compensation has worked, select each **Filter Comb** and compare the **Raw Data** and **Compensated Data** graphs. The Compensated Data graph should show only samples containing the relevant dye in the designated channel* (**Figure 16-Figure 21**).

> **Save CC Object** > Name the file "**SpeeDx 20ul**" for 20 µl reaction or "**SpeeDx 10ul**" for 10 µl reaction

Figure 16. Raw Data and Compensated Data for Filter Comb 440-488 (LC480 II)

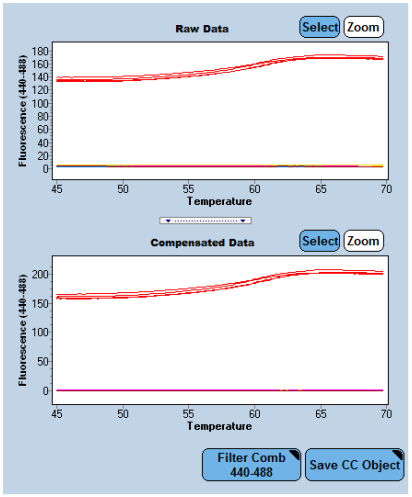


Figure 17. Raw Data and Compensated Data for Filter Comb 465-510 (LC480 II)

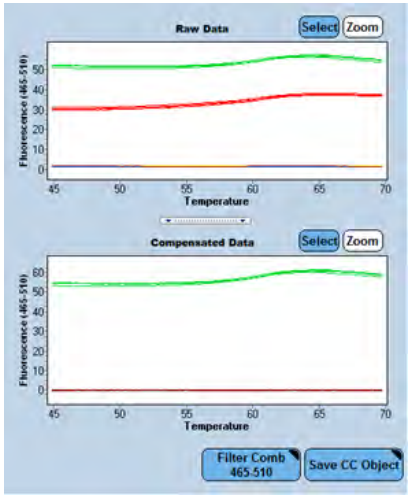


Figure 18. Raw Data and Compensated Data for Filter Comb 533-580 (LC480 II)

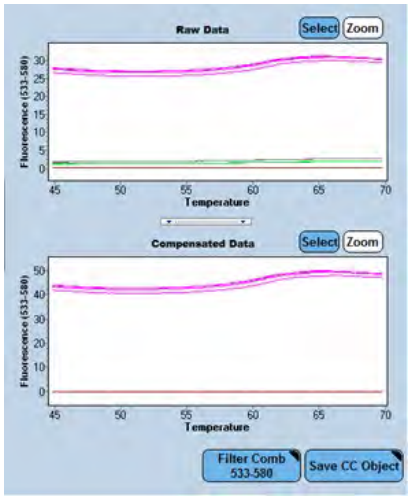


Figure 19. Raw Data and Compensated Data for Filter Comb 533-610 (LC480 II)

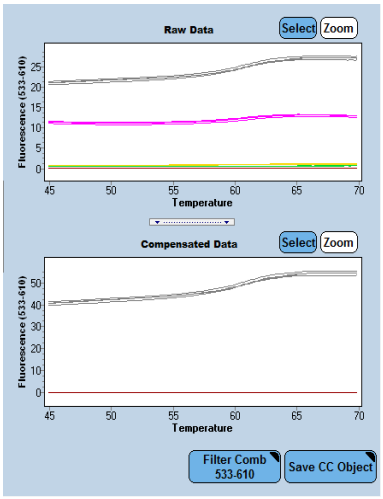


Figure 20. Raw Data and Compensated Data for Filter Comb 533-640 (LC480 II)

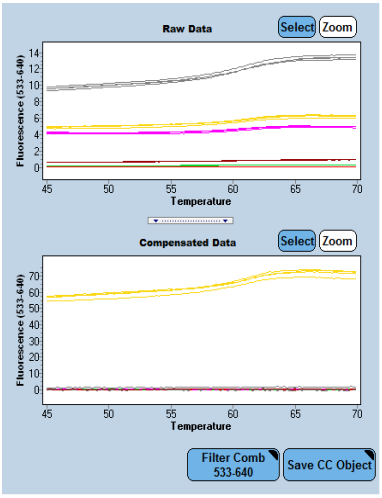
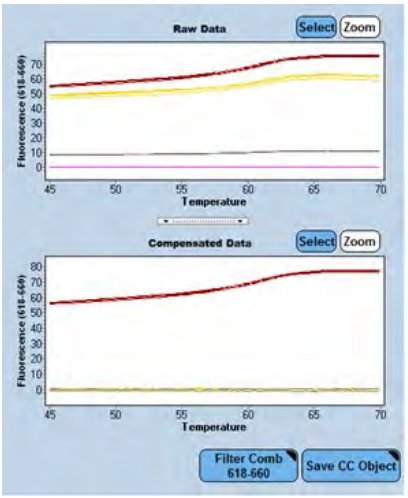


Figure 21. Raw Data and Compensated Data for Filter Comb 618-660 (LC480 II)



12 Appendix B: Colour Compensation for 10 µl reaction volume

The ***Plex****PCR*® Colour Compensation kit can be performed at a final reaction volume of 20 µL on a 96 well plate, or at a final reaction volume of 10 µL on a 384 well plate. Refer to **Section 8.1** for instructions for performing 20 µL reactions on a 96 well plate.

12.1 Preparation of Master Mixes for 10 µl reaction volume on a 384 well plate

Note: Before use of reagents, thaw completely, and mix thoroughly by briefly vortexing

To create the CC file, 3 replicate reactions of each reporter dye (6 dyes) are required. In addition, 3 replicate control reactions with no dye, are also run. Therefore a total of 7 mixes (6 dyes and 1 with no dye), each with 3 replicates are required.

The following outlines a protocol to make CC Master Mixes (7 mixes) which contains components required for qPCR as well as addition of CC templates. The mixes for the 3 replicate reactions are made in excess (extra 2 reactions for total of 5 reactions).

More specifically, each reaction has a 10 µl final volume, composed of 7 µl Master Mix A (**Table 15, *Plex*** Mastermix), 2.5 µl CC template, and 0.5 µl each reporter dye CC Mix (**Table 17-Table 22**), or 0.5 µl of water (**Table 16**). This is directly plated into the PCR plate and run on the qPCR instrument.

Seal plate, centrifuge the plate and transfer to thermocycler.

Note: Do NOT store excess mixes

Table 15. Master Mix A for 10 µl reaction volume

Reagent	Concentration	Volume (for 37 reactions)
Plex Mastermix (**BLUE**)	2x	185 µl (37 x 5 µl)
Nuclease Free Water (**LIGHT BLUE**)	N/A	74 µl (37 x 2 µl)
Total volume (µl)		259 µl (37 x 7 µl)

Table 16. CC Master Mix (BLANK)

Reagent	Concentration	Volume (5 reactions)
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Nuclease Free Water (**LIGHT BLUE**)	N/A	2.5 µl (5 x 0.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

Table 17. 488 CC Master Mix

Reagent	Concentration	Volume (5 reactions)
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
488 CC Mix (**BROWN**)	20x	2.5 µl (5 x 0.5 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

Table 18. 510 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
510 CC Mix (**GREEN**)	20x	2.5 µl (5 x 0.5 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

Table 19. 580 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
580 CC Mix (**YELLOW**)	20x	2.5 µl (5 x 0.5 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

Table 20. 610 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
610 CC Mix (**PURPLE**)	20x	2.5 µl (5 x 0.5 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

Table 21. 640 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
640 CC Mix (**WHITE**)	20x	2.5 µl (5 x 0.5 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

Table 22. 660 CC Master Mix		
Reagent	**Concentration**	**Volume (5 reactions)**
Master Mix A (**Table 15**)	N/A	35 µl (5 x 7 µl)
660 CC Mix (**BLACK**)	20x	2.5 µl (5 x 0.5 µl)
CC template (**NEUTRAL**)	N/A	12.5 µl (5 x 2.5 µl)
Total volume (µl)		50 µl (5 x 10 µl)
Load 10 µl onto PCR plate		

12.2 Programming and analysis

Details for programming and analysis are described in **Section 10-11**.

12.3 Applying the PlexPCR® CC file

The ***Plex****PCR*® CC file can be subsequently applied to all ***Plex****PCR*® kits or ***Resistance****Plus*® kits run files generated on the same instrument at the same reaction volume. Please refer to the ***Plex****PCR*® CC kit IFU or Product specific IFU for the details on how to apply the ***Plex****PCR*® CC file.

13 Customer and technical support

Please contact Technical Support for questions on reaction setup, cycling conditions and relevant data.

Tel: +61 2 9209 4169, Email: tech@speedx.com.au

14 Glossary

European Conformity
For *In Vitro* Diagnostic Use

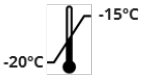
Catalogue number

Batch code

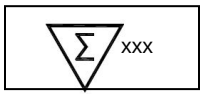
Authorised Representative
In the European Community

Manufacturer

Date of manufacture



Temperature limitation



Contains sufficient for xxx determinations

Use by Date