I.DOT

User Manual

Applies to I.DOT Assay Studio software version 1.9.1

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Content

1. Preface

Purpose

This user manual details specific information pertaining to safe and proper handling and operation of the I.DOT low volume non-contact dispensing system and I.DOT consumables.

Conventions Used

The following conventions are used throughout this manual and are described below for your reference:

	This note type indicates that a potential hazard to your personal safety, or potential for equipment damage exists.
	This note type presents essential content indicating that there is a potential for assay failure, diminished data quality, and/or a loss of data if the information presented is ignored.
	Information contained in a tip may offer helpful suggestions, alternative procedures, methods and/or shortcuts.
Bold	When appearing in text or in a procedure, the bold text serves to highlight a specific button, key stroke, or menu option available.
Italic	Used to emphasize an important word or expression within the text. Formatting of a book title, journal, or other documentation Used to indicate the special or unusual meaning of a word or phrase.

2. Setting Up Dispensing Runs

This chapter describes how to set up the target and I.DOT source plates, prepare the instrument, start and analyze a dispense run.

When opening the *I.DOT Assay Studio* software, it will ask to open a saved protocol or create a new protocol.

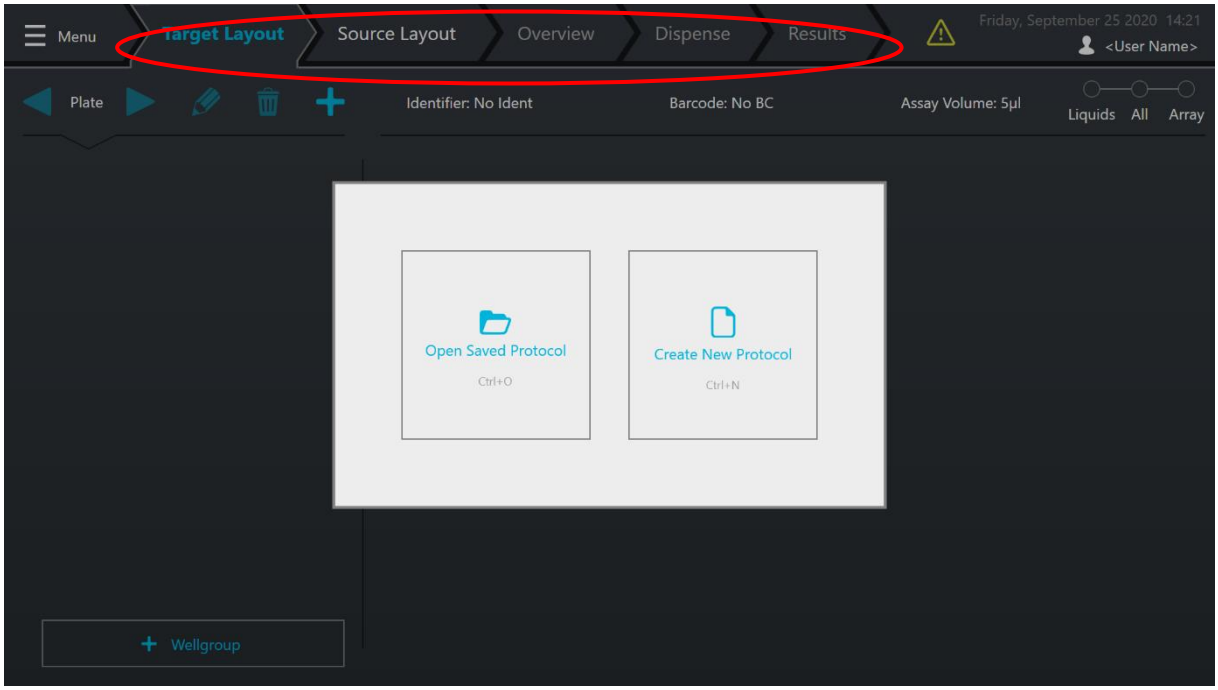


Figure 1: Starting screen of I.DOT Assay Studio software.

The **top bar** of the software screen (Figure 1, red circle) guides through each single step of a dispense run, highlighting the current step in blue colour.

2.1. Protocol Settings Setup

Choose Create New Protocol and the Protocol Settings are shown (Figure 2).

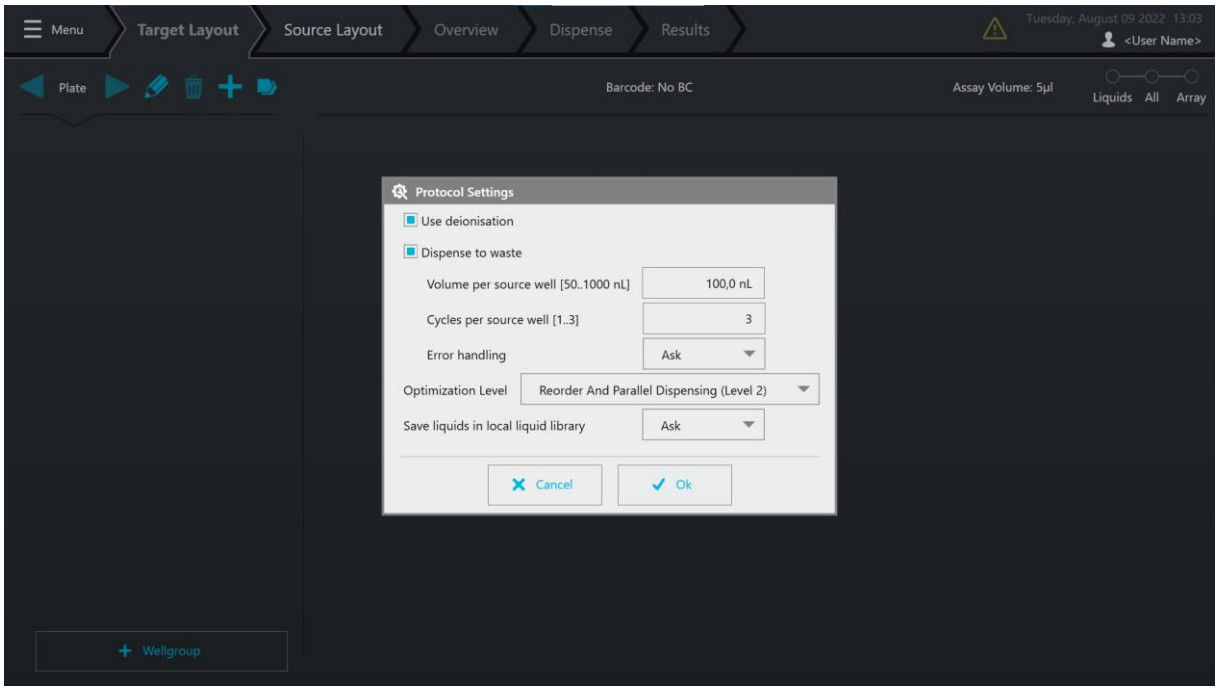


Figure 2: Protocol Setup screen.

Following items can be edited:

- Enable/disable the deionisation function.
- Enable/disable the Dispense to waste function (priming of source wells, to make sure there are no air bubbles directly above the orifice).
    - Adjust the volumes for priming of each source well.
    - Adjust the number of priming cycles for each source well.
- Edit the error handling if no droplets are detected during priming.
- Edit the optimization level (reducing dispensing time by reordering dispensing steps and use parallel dispensing).
- Edit Save liquids in local liquid library.

Press "Ok" to proceed with the definition of the Target Layout setup.

> Protocol settings can be changed any time by clicking on "Menu > Settings > Protocol settings".

2.2. Target Layout Setup

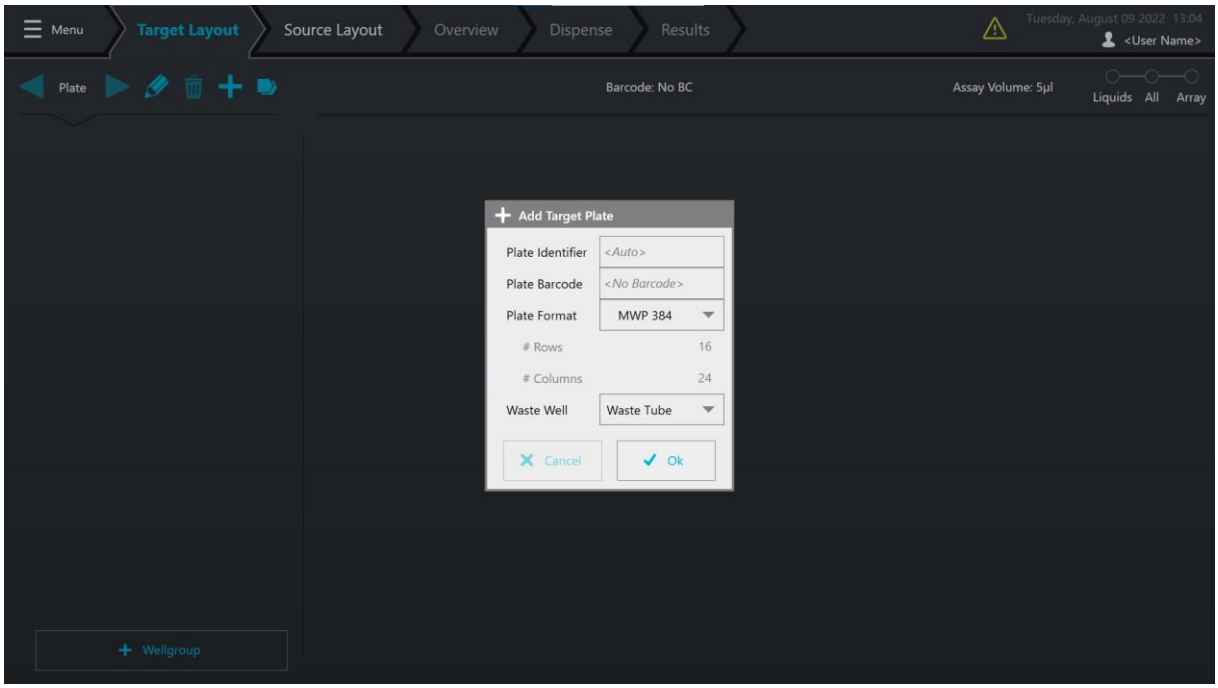


Figure 3: Protocol Setup screen.

Following items can be edited:

- Optional: **Enter** a **Plate Identifier**.
- Optional: **Enter Plate Barcode** ID.
- **Select** the **target plate format**.
- Optional: **Select** a **Waste Well** position if waste dispense is enabled.

Press "**Ok**" to proceed.

☞	Target plate properties can be edited any time by **clicking** on the  **button** in the top-left corner of the **Target Layout** window.

☞	The barcode reader is available in all devices and can be enabled in the **Advanced Device settings** (see chapter 5.4. About Advanced Device Settings). The barcode of the source and target plate can be scanned, as long as the barcode is on the left side of the plate. Click on  button to scan.

Select wells which should receive liquids by clicking on the desired well positions shown in the *Target Layout* tab of the main menu. Selected wells are highlighted in blue colour and are defined as one Wellgroup (Figure 4).

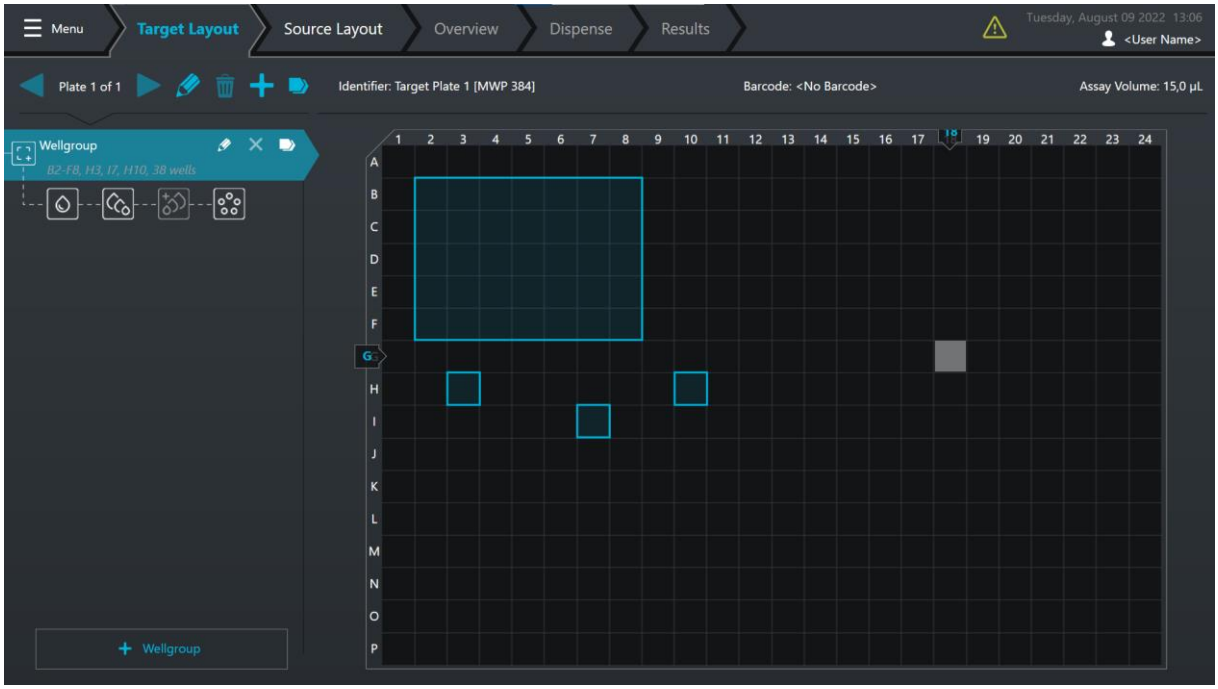


Figure 4: Target layout screen with blue highlighted well group.

Additional target plates can be added in two ways:

- Clicking on the button to get a new empty target plate layout
- Clicking on the button to duplicate the target plate layout. In the popup that shows up it can be selected which Target Plate layout should be duplicated and if it should be duplicated to a New target plate, to a New protocol or to an Existing protocol (Figure 5).

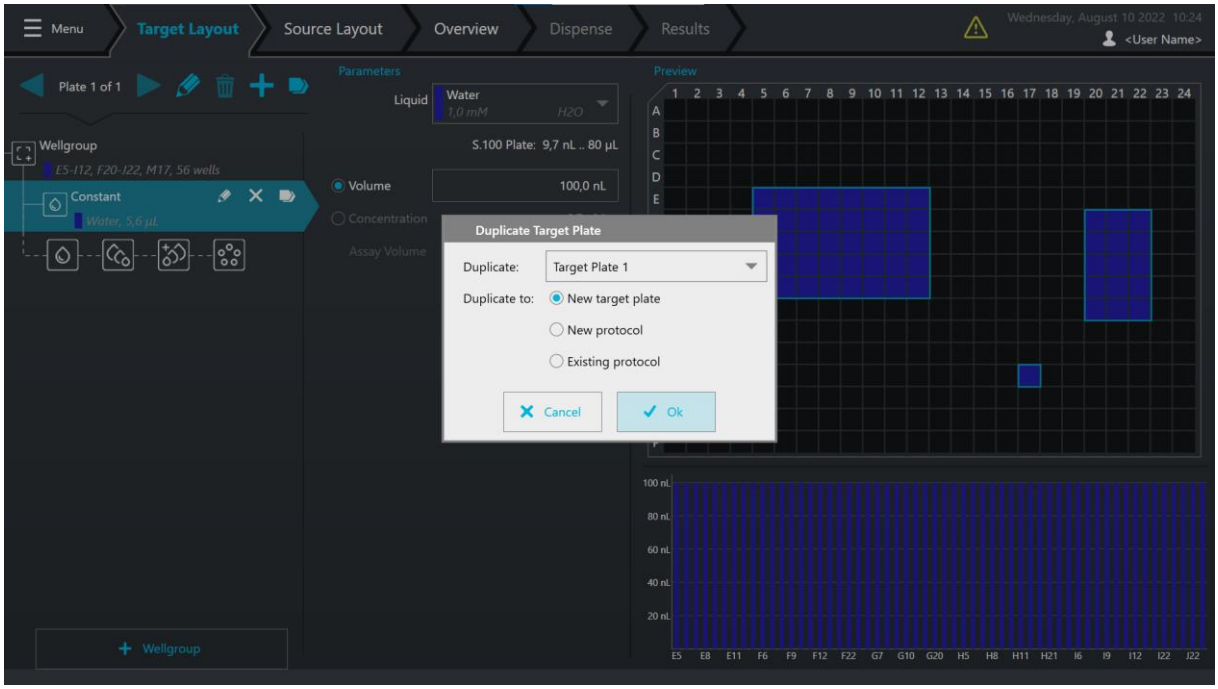


Figure 5: Duplicate Target Plate popup

The arrow buttons enable switching between the different target plate layouts.

2.2.1. Dispensing Options

The *I.DOT Assay Studio* software offers 4 different dispensing options, which are indicated as buttons , , and on the left below the well group (Figure 6, red circle).

"Constant" is a simple liquid transfer step to dispense a distinct volume or concentration of a chosen liquid to the selected wells on the target plate.

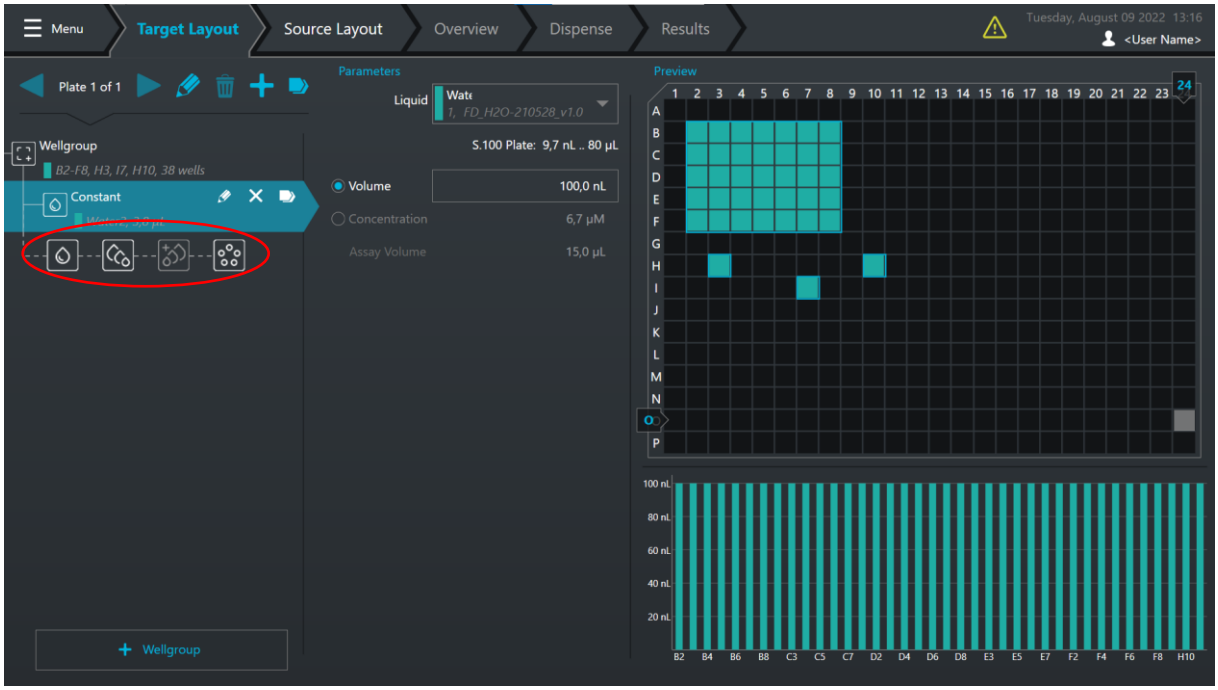


Figure 6: Dispensing option "Constant".

When performing dispensing runs by **concentration**, the I.DOT Assay Studio is able to calculate the required volume, as long as all **parameters** (**total assay volume, liquid stock concentration** and desired **concentration in target** well) are given.

Total assay volume can be defined for the **whole target plate** by clicking on the button and adjusting the **assay volume**.

Total assay volume can also be different for several well groups. When defining the dispensing option by concentration, click on **Uncouple** to define **another total assay volume** for the **selected well group**, which is different to the total assay volume for the whole target plate.

: “Dilution” creates direct dilutions within the selected well group.

Choose the liquid and desired volume or concentration that shall be dispensed.

Figure 7: Dispensing option "Dilution".

Following items can be edited:

- Volume or concentration: Set volume/concentration start and end point for *Logarithmic Auto* and *Linear distribution*. Set starting volume/concentration for *Custom distribution*.
- Add additional “0” step to dilution series.
- Distribution: Choose distribution type of the dilution series. Linear, logarithmic, and individual dilution factors can be applied.
- Add replicates.
- Change dilution pattern to horizontal or vertical distribution.

“Normalization” equalizes filling level of each target well to the same volume within a dilution series.

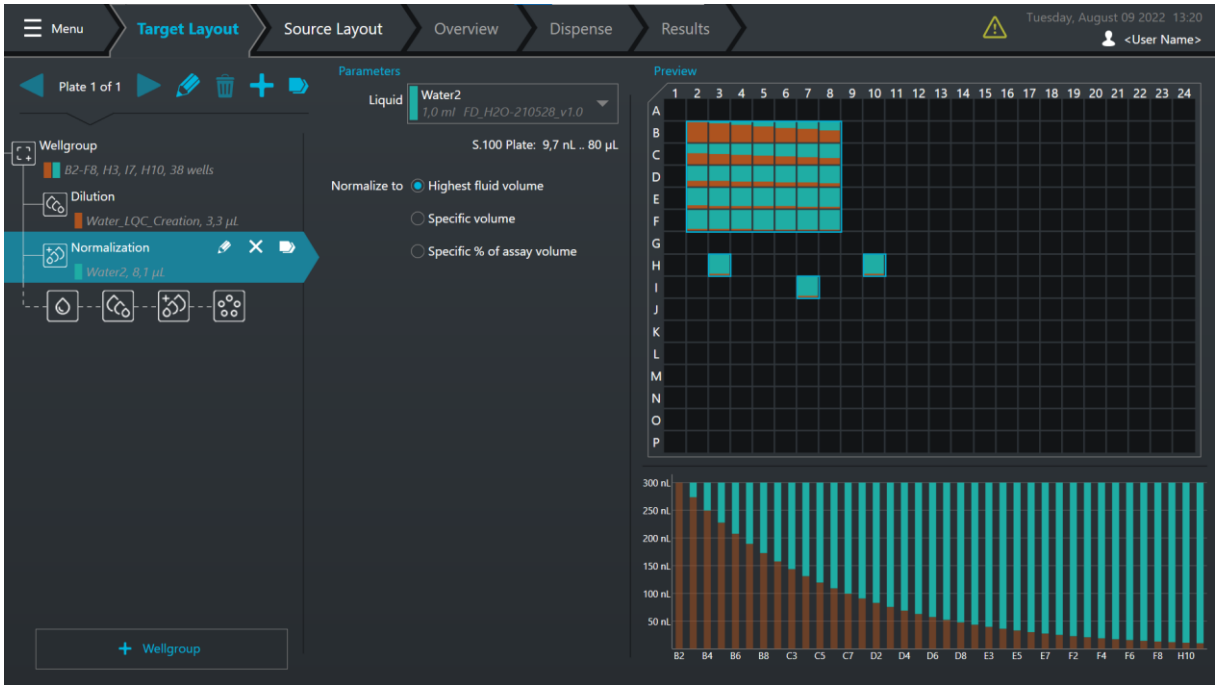


Figure 8: Dispensing option "Normalization".

Select one of the normalization options:

- Highest fluid volume: Wells are equalized to the same total volume using the well with the highest volume level of the well group.
- Specific volume: Equalize wells to a user specific volume.
- Specific % of assay volume equalizes well volume to a desired percentage of the total assay volume.

 “Array” creates arrays within one or multiple wells.

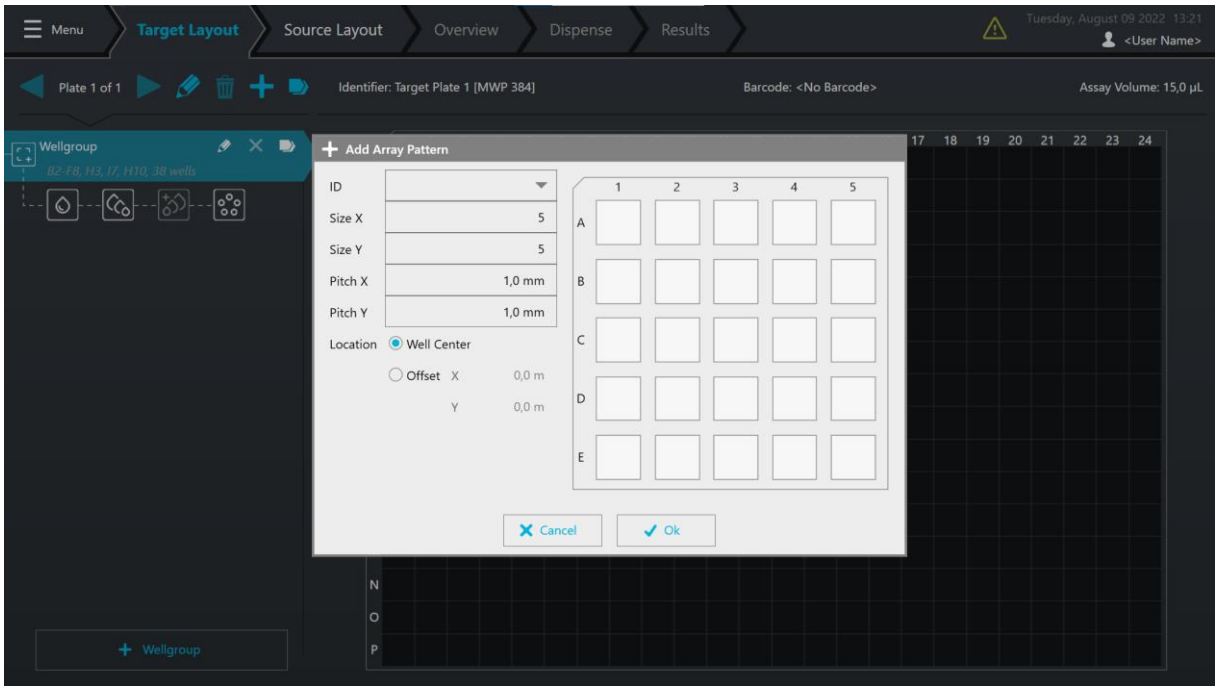


Figure 9: Dispensing option "Array"-Setup screen for array size and position.

Following items can be edited:

- ID: Select a pre-defined array or create a new one.
- Size X/Y: Edit array size (spot number).
- Pitch X/Y: Edit distance between spots.
- Location: Select position of array within well.

Choose wells to be dispensed and desired dispensing option (Figure 10).

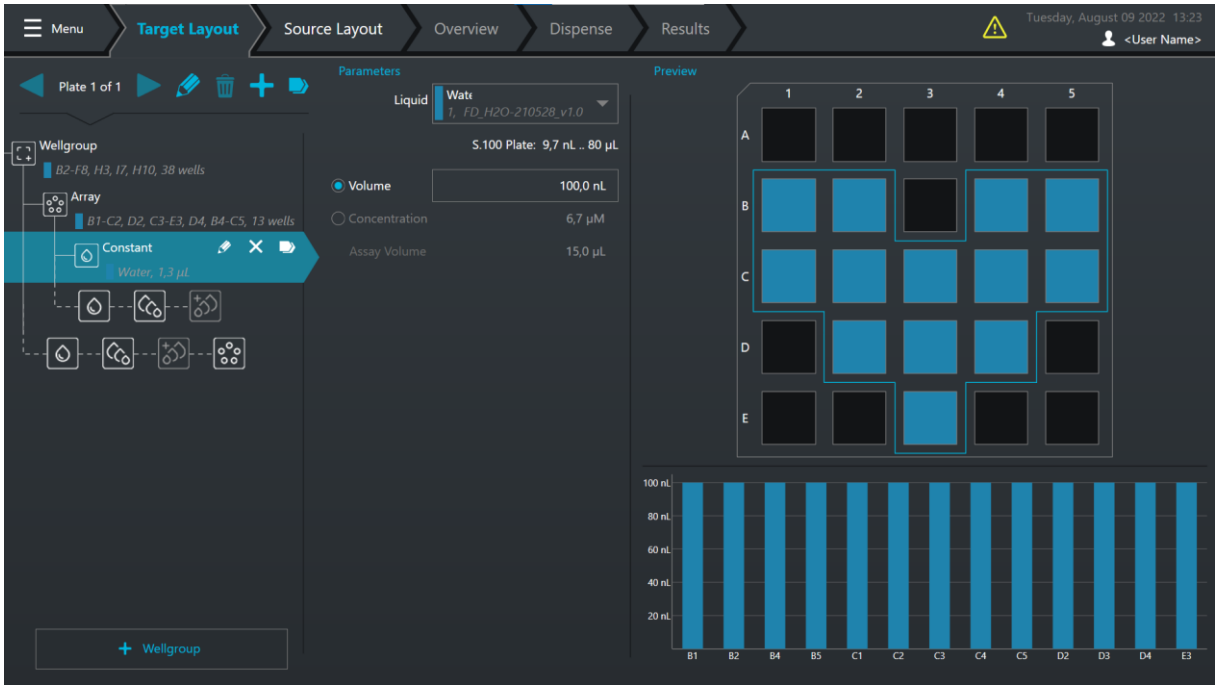


Figure 10: Defining dispensing options within an array.

Selected array pattern will be applied to all chosen wells of target layout.

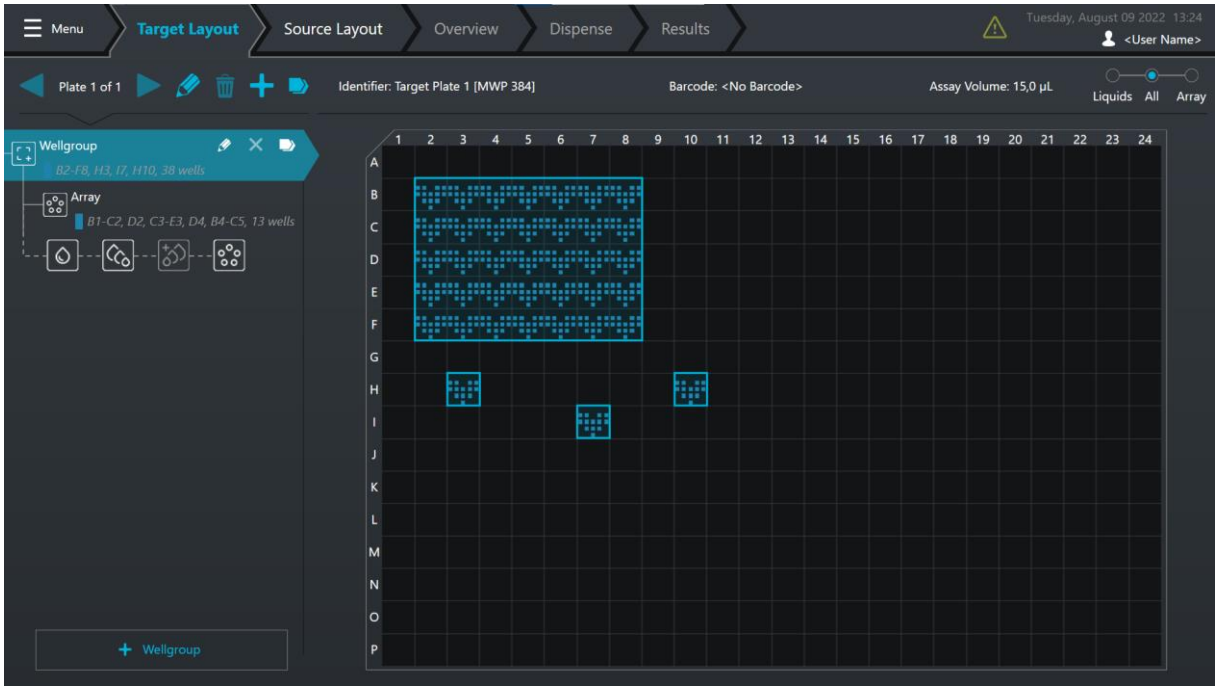


Figure 11: Final array in the target layout.

Please note: *Mixing*, *Dilution*, *Normalization* and *Array* will affect **all** wells of your selected area in the respective well group.

2.2.2. Edit Well groups

To edit a well group, select the respective well group in the list on the left side of the screen. Click into the chosen well group on the target plate layout that shall be edited, blue squares in each corner of the area will appear.

Change the size of an area by hovering on the blue squares until they turn red, click and hold to drag to the desired size.

Change the position of the whole well group by clicking into the middle of the well group, click and hold to drag to the desired position on the target plate.

Remove selected wells by dragging them to the bin or pressing the "Del" key on the keyboard (Figure 12).

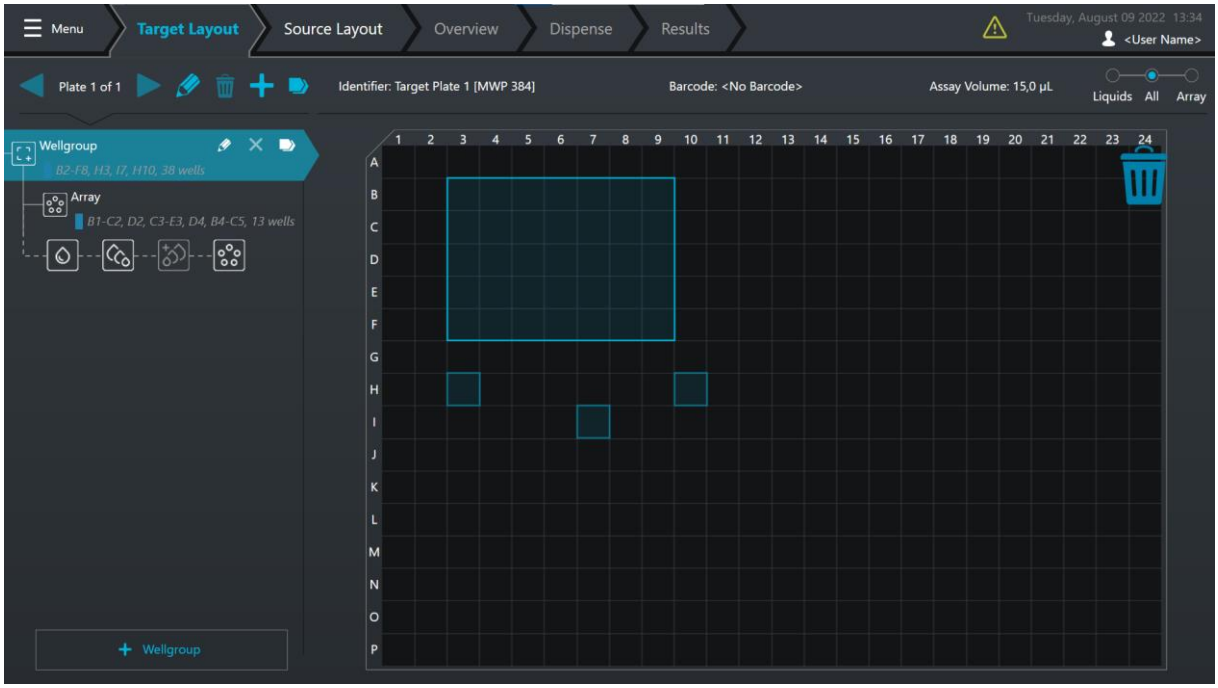


Figure 12: Assigning target wells.

2.2.3. Copy Well groups

Whole well groups with already defined dispensing option, volume and liquid can be copied by clicking on the button of the respective well group. Select the liquid you want to use and the location for the new well group (Figure 13).

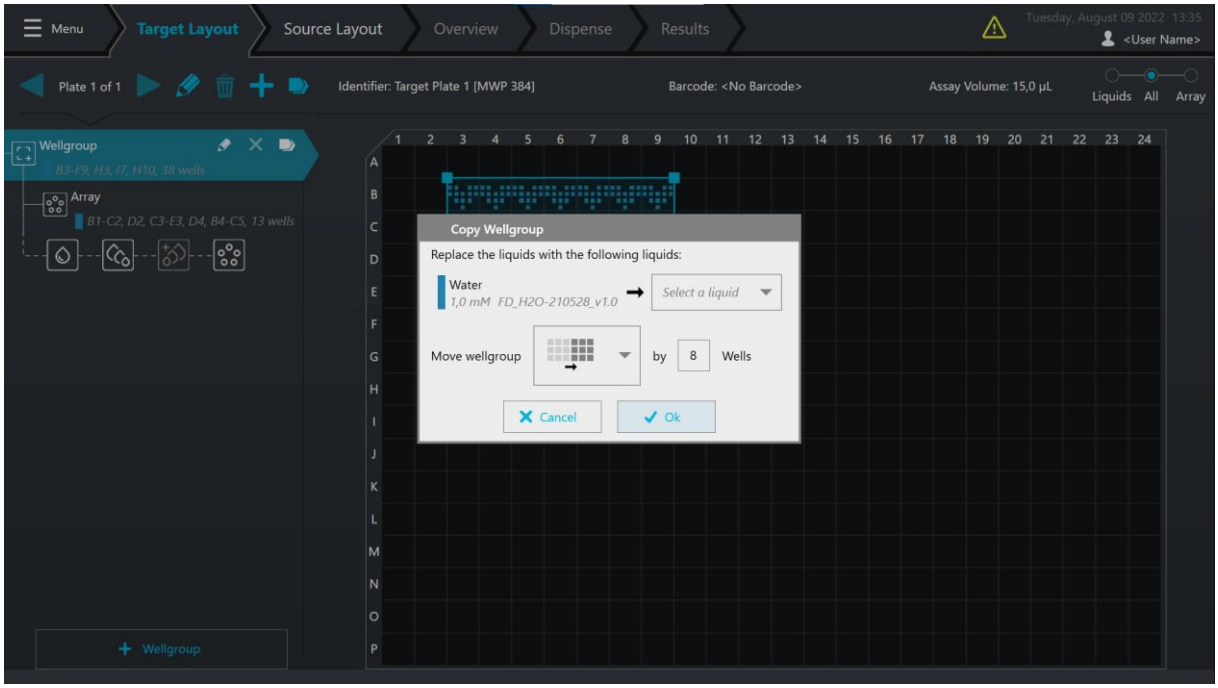


Figure 13: Copy well group-Setup screen.

2.3. Source Plate Setup

Once the target layout is defined, move to Source Layout in the top bar to proceed with the protocol.

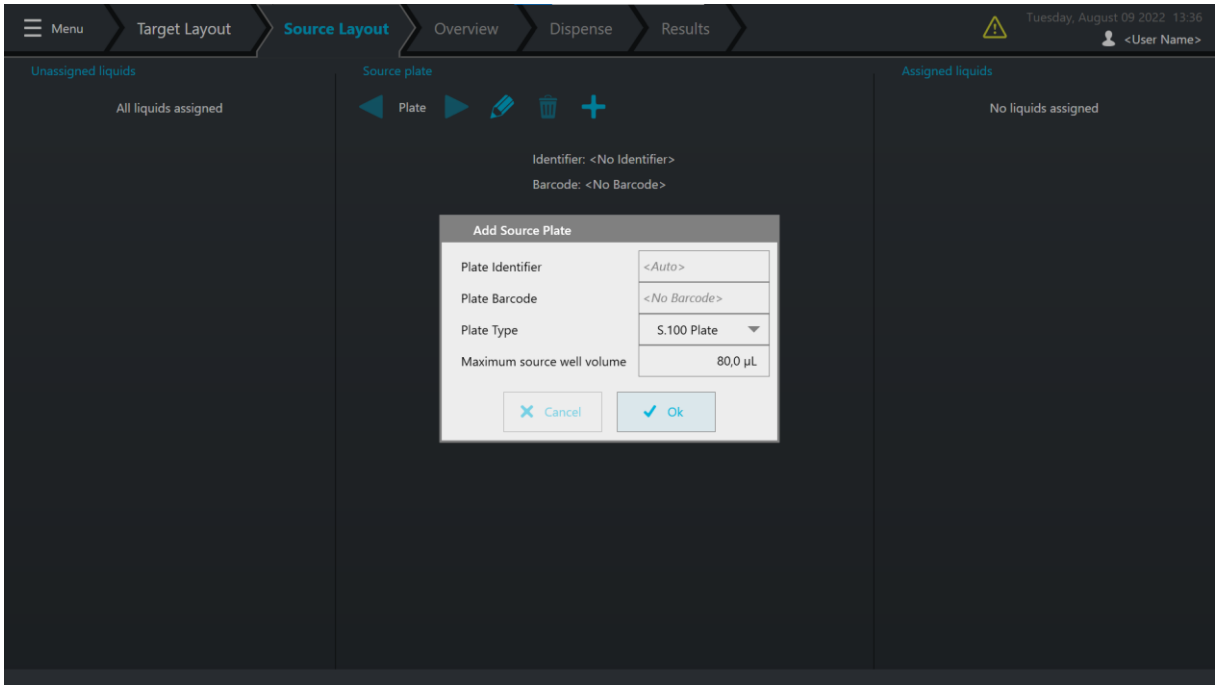


Figure 14: Source Layout-Setup screen.

Following items can be edited:

- Optional: **Enter** a **Plate Identifier**.
- Optional: **Add** the **Plate Barcode** ID.
- **Select** the source **Plate Type**. For detailed description of the different source plate types, see chapter 5.1.2. Liquid Class Library.

Press "**Ok**" to proceed.

☞	Source plate properties can be edited any time by **clicking** on the **button** in the *Source Layout* window. Additional source plates can be added by **clicking** on the **button** on the top side of the *Source Layout* window.

All liquids and the **minimum required volumes** for dispensing used in the protocol are listed on the left side of the screen in the *Unassigned liquids* section (Figure 14).

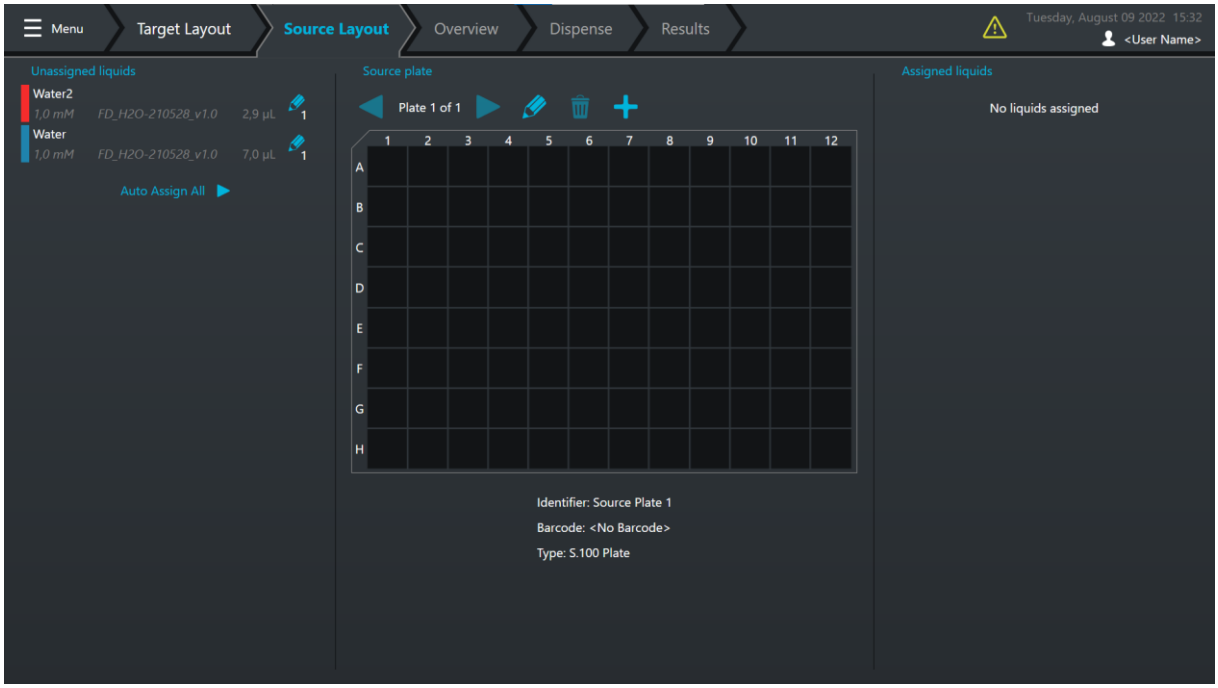


Figure 15: Source Layout-Setup – Unassigned Liquids.

Liquids are assigned either by manually dragging them to the desired position on the source plate or automatically by clicking on Auto Assign All. Assigned liquids move from the left to the right side of the screen into the *Assigned liquids* section.

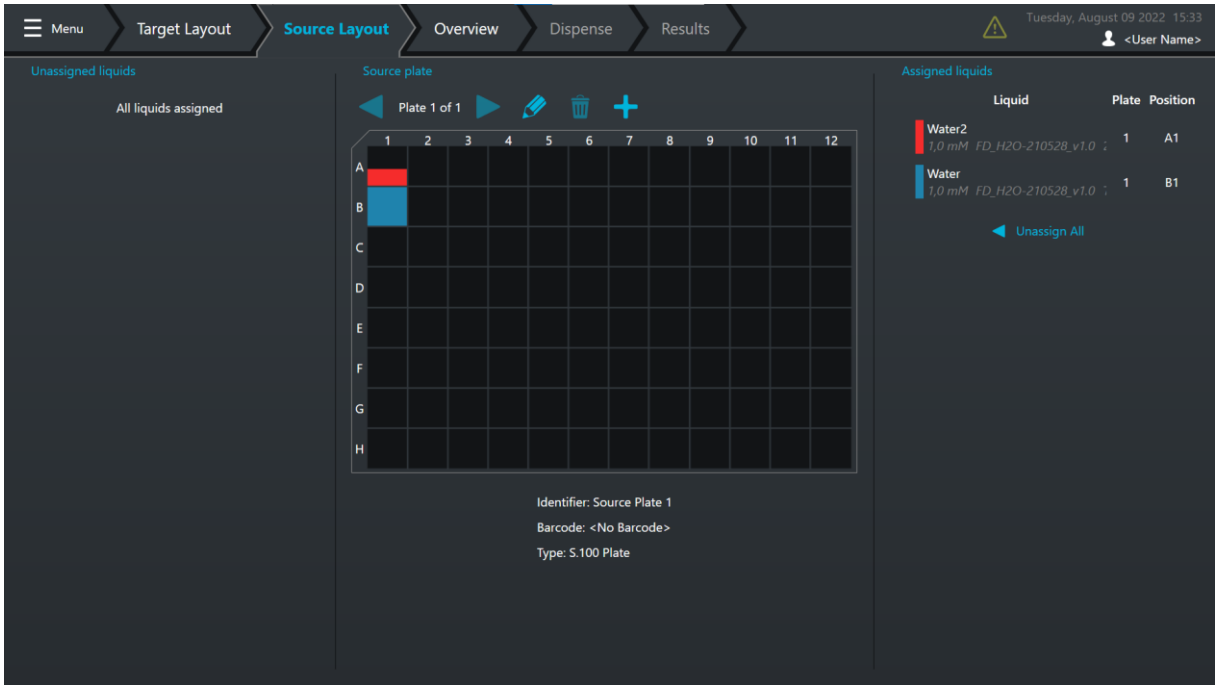


Figure 16: Source Layout-Setup - Assigned liquids.

When assigning the liquids **manually**, each liquid can get assigned **separately** by dragging them to the desired position or a **selection of liquids** can get assigned together by marking them and dragging them together to the desired position (Figure 17).

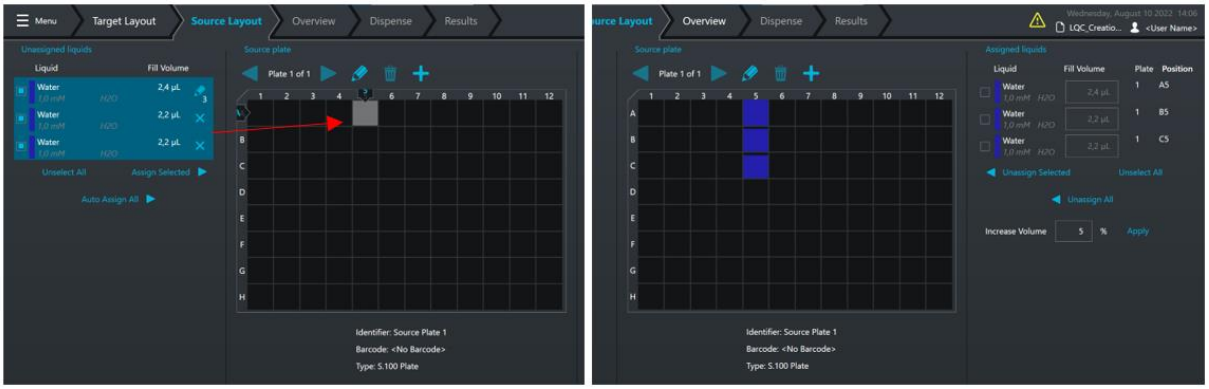


Figure 17: Multi-select feature for assigning liquids.

The initial fill volume of each well can be changed by increasing the fill volume by a certain percentage or manually by typing in the desired fill volume to prevent the wells from running empty or to account for additional dead volume. Therefore, one or several wells can be

selected. Changing the fill level manually only one of the selected wells needs to be changed. It will be applied to all selected wells.

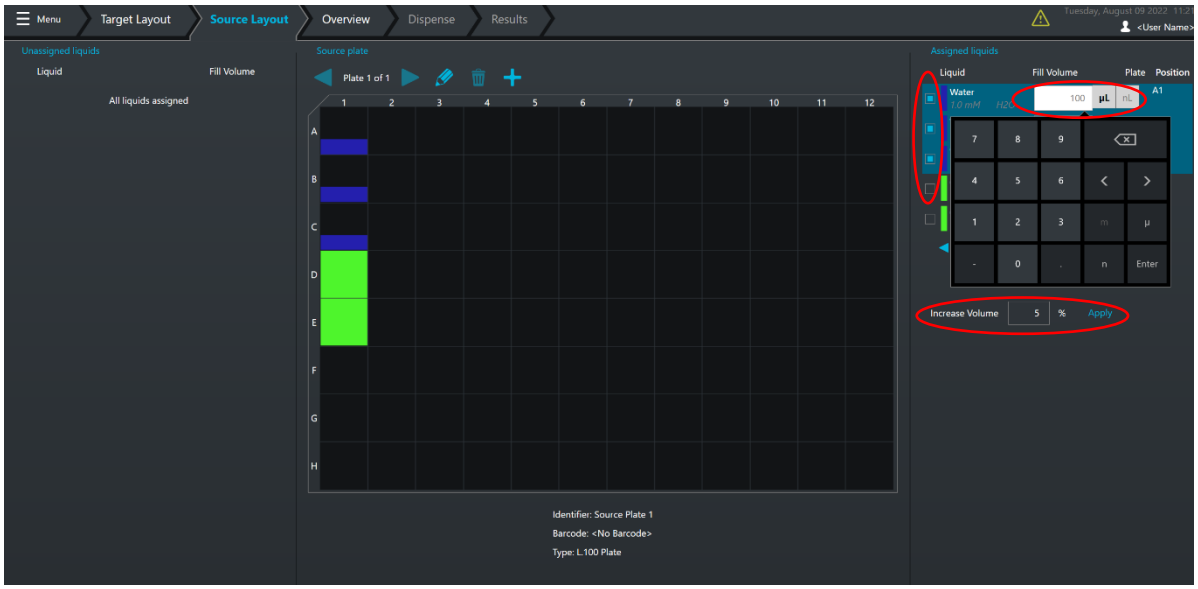


Figure 18: Source plate setup. Change fill volume

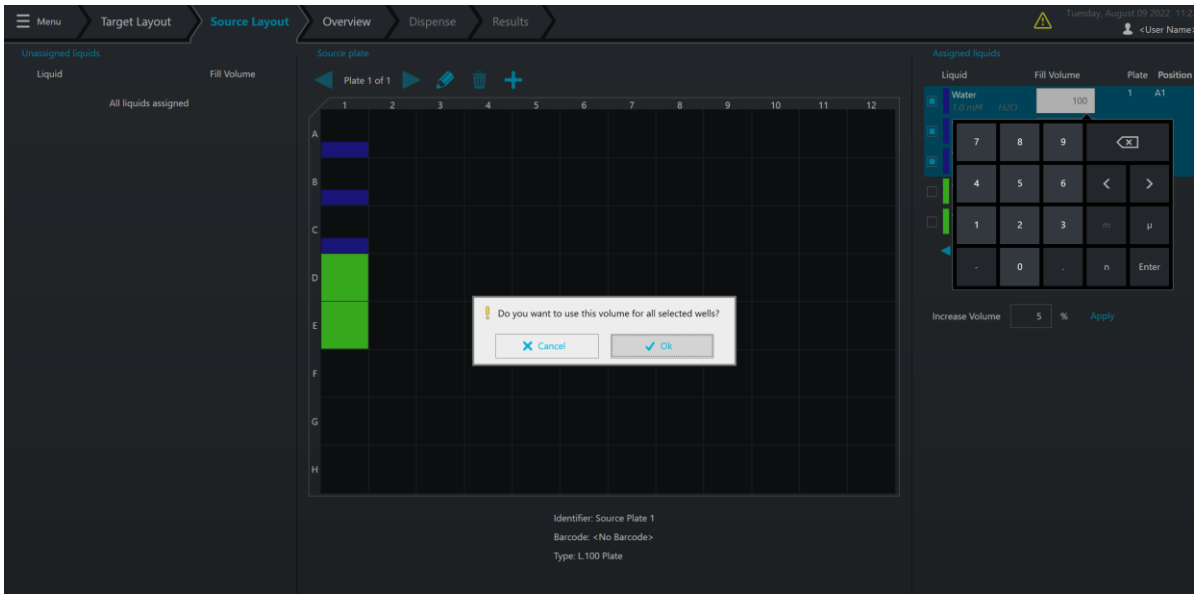


Figure 19: Source plate setup. Change fill volume

After defining the source plate layout click on Overview in the top bar to proceed.

2.3.1. Enable source liquid duplicate popup

Enabling the source liquid duplicate popup (Figure 20, red circled) allows you to define a number of source wells for the respective liquid in the source layout setup.

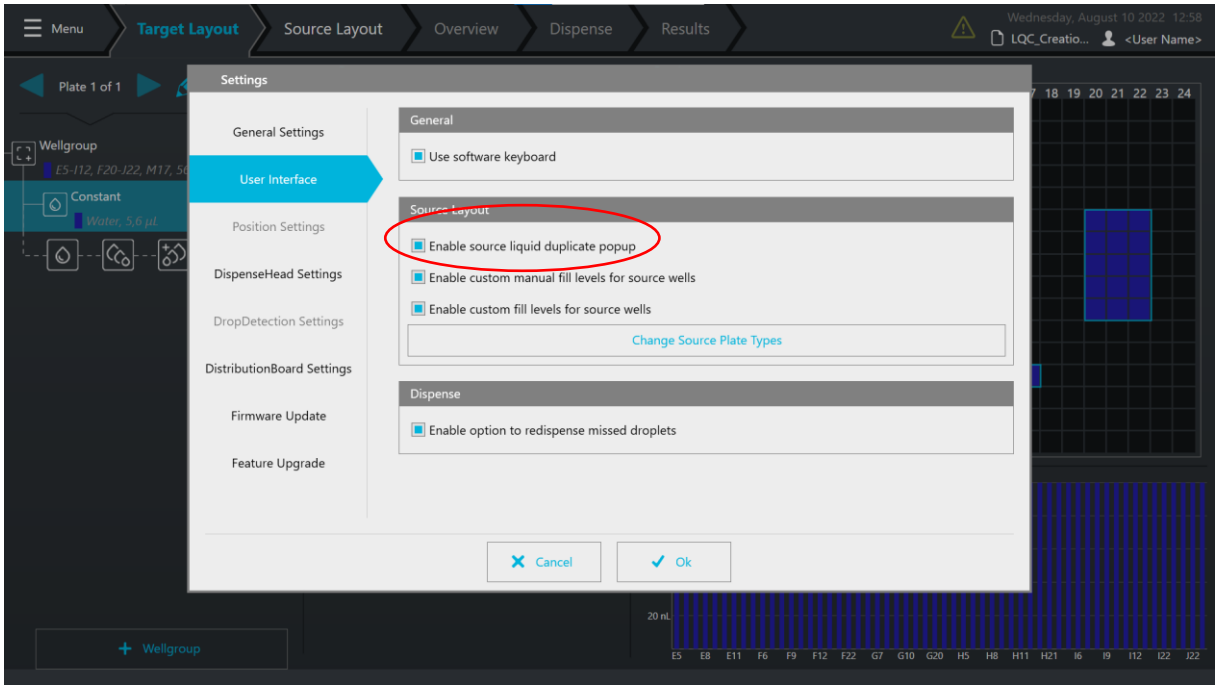


Figure 20: User interface settings - Enable source liquid duplicate popup.

When the liquids are still shown in the Unassigned liquids section, clicking on the  button right next to the liquid opens a popup window (Figure 21). The value written there is the number of source wells, the volume of the respective liquid gets averaged in.

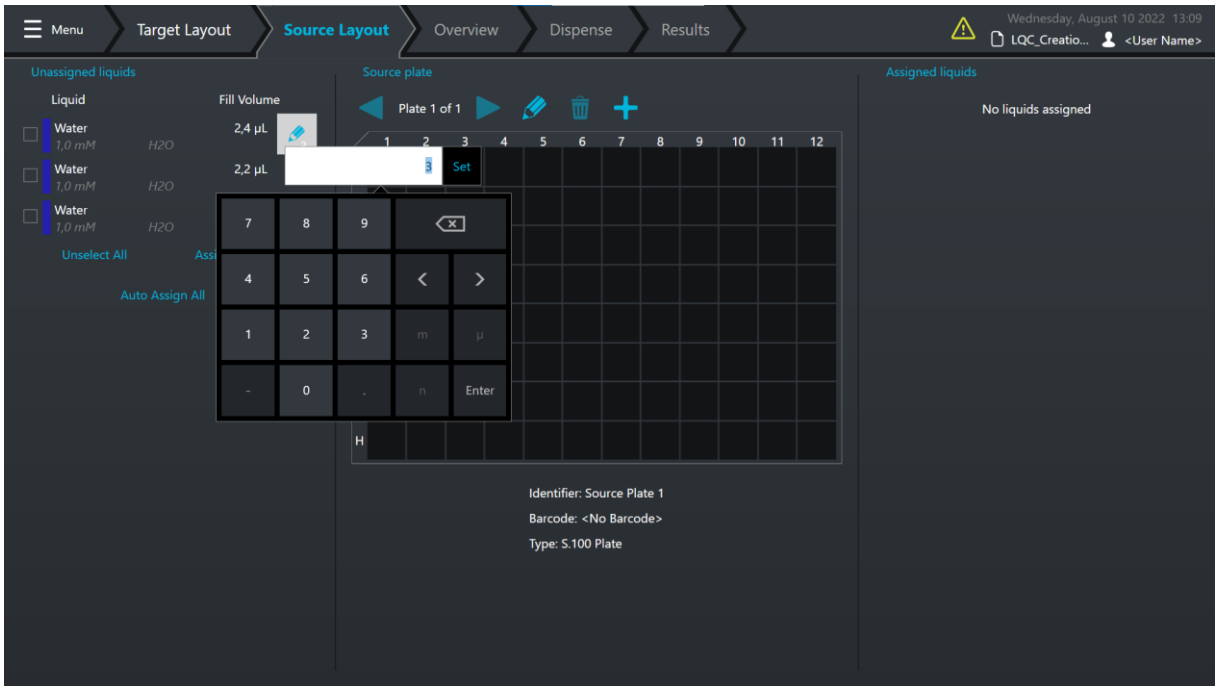


Figure 21: Source liquid duplicate popup.

2.4. Overview

The **Overview** displays a simulation mode, in which protocols can be reviewed. The time required to dispense the whole protocol as well as the total number of dispensing steps are shown. By clicking on **Start Simulation** all dispensing steps can be monitored. Adjusting the simulation speed enables **speeding up** or **slowing down** the simulation, whereas adjusting the dispensing step allows to **observe specific steps** of the protocol.

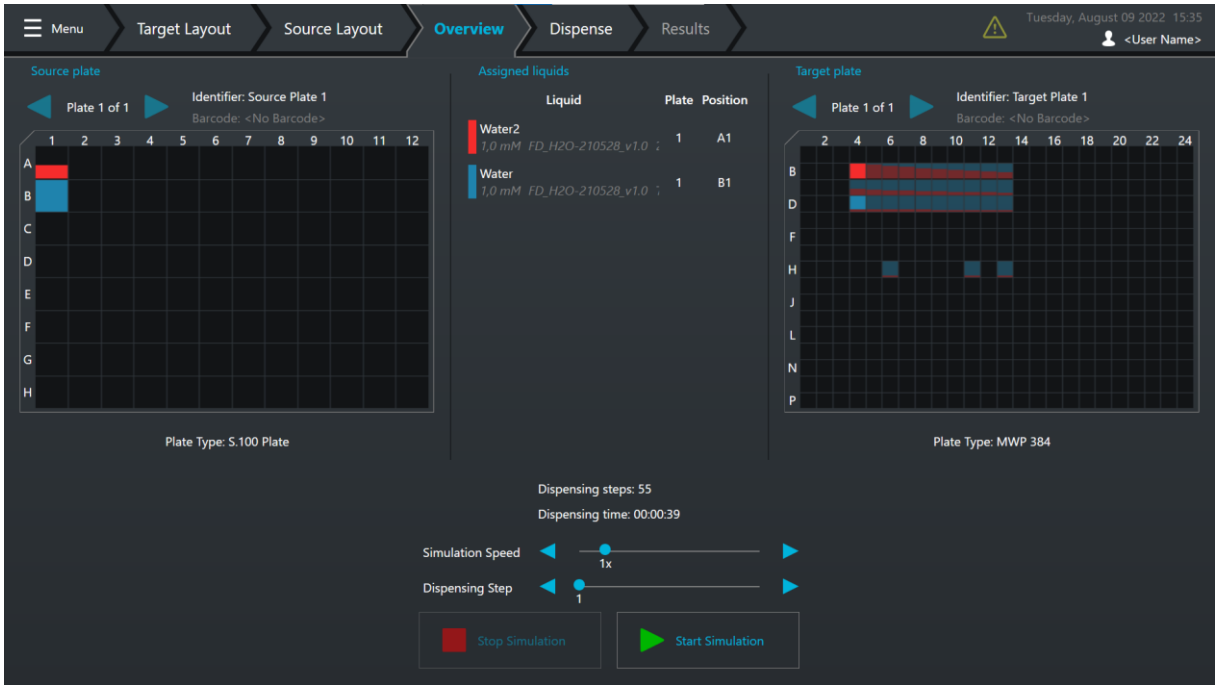


Figure 22: Overview – Simulation mode screen.

Click on **Dispense** in the top bar to proceed.

2.5. Dispense

As soon as the dispensing protocol is set up, **prepare** the I.DOT source plate with respective liquids.

Click on **Start Dispensing** and the software asks to **insert the source plate** into the upper tray and the **target plate** into the lower tray of the device.

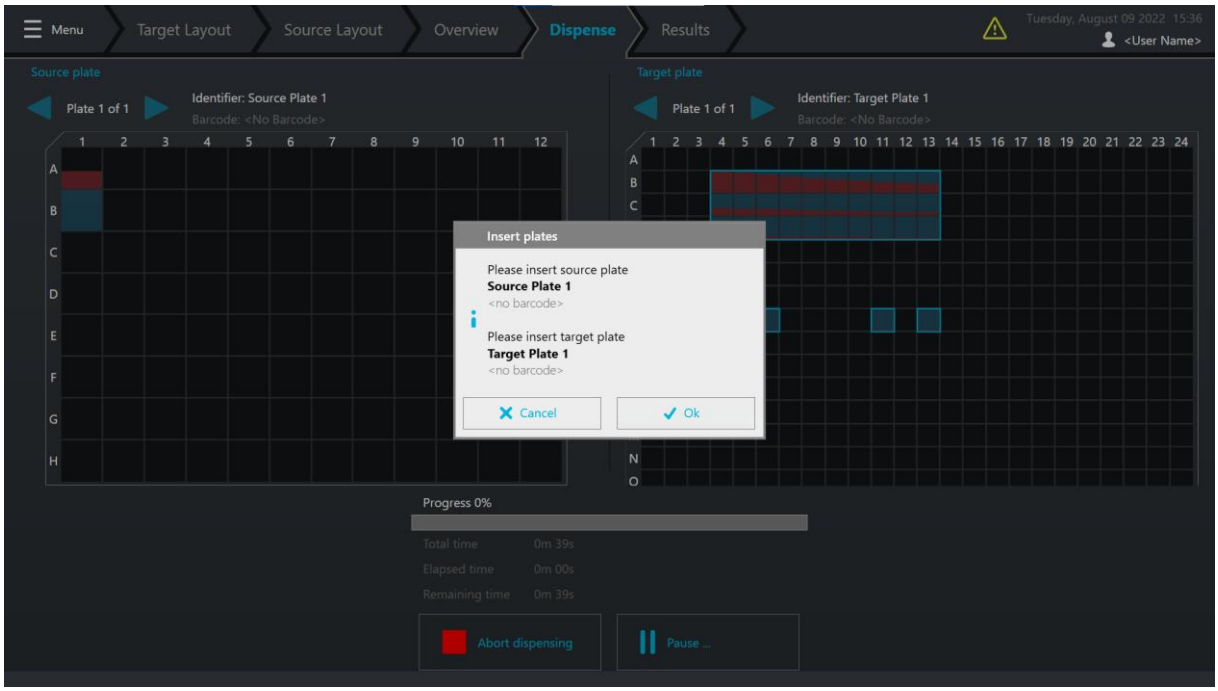


Figure 23: Dispense screen – dispense a protocol.

Dispensing runs can be paused at any time of the run.

Dispensing runs can be aborted any time by **clicking** on **Abort Dispensing**.

NOTE: Source wells might have to be refilled with liquid and target plate has to be exchanged for repetition of the dispensing run.

After the run is finished, a pop-up window showing "Dispensing completed" appears. Clicking on **Ok** will automatically open the results tab.

2.6. Re-dispense Missed Droplets

If there are any droplets missing by drop detection during the dispense run a Missed Droplets window shows up directly after the dispense run. The information on the popup shows in which dispense steps droplets were missing. By selecting the wells and **clicking Re-dispense Missed Droplets**, the I.DOT will fill up these target wells according to the counted missed droplets.

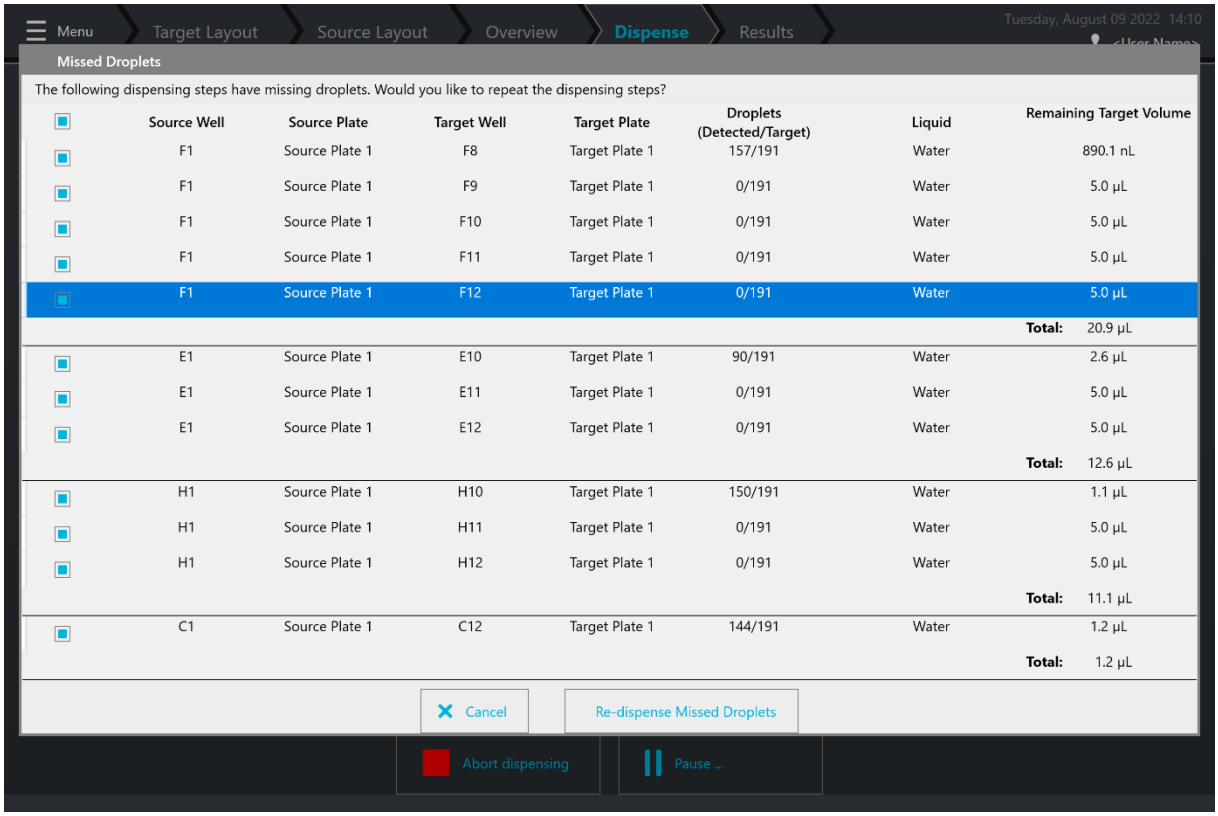


Figure 24: Missed Droplets window.

2.7. Results

The results tab shows the **performance of the dispensing run** directly after distributing all liquids into the target plate. All **green** wells received the **expected volume** counted in number of droplets, whereas all **red** wells are **missing liquids**.

All used liquids are displayed on the left side of the screen with the corresponding position on the source plate. The software provides information on the **performance** of each source well.

By clicking on any target well, all liquids and volumes dispensed into this specific well are shown on the right side of the screen.

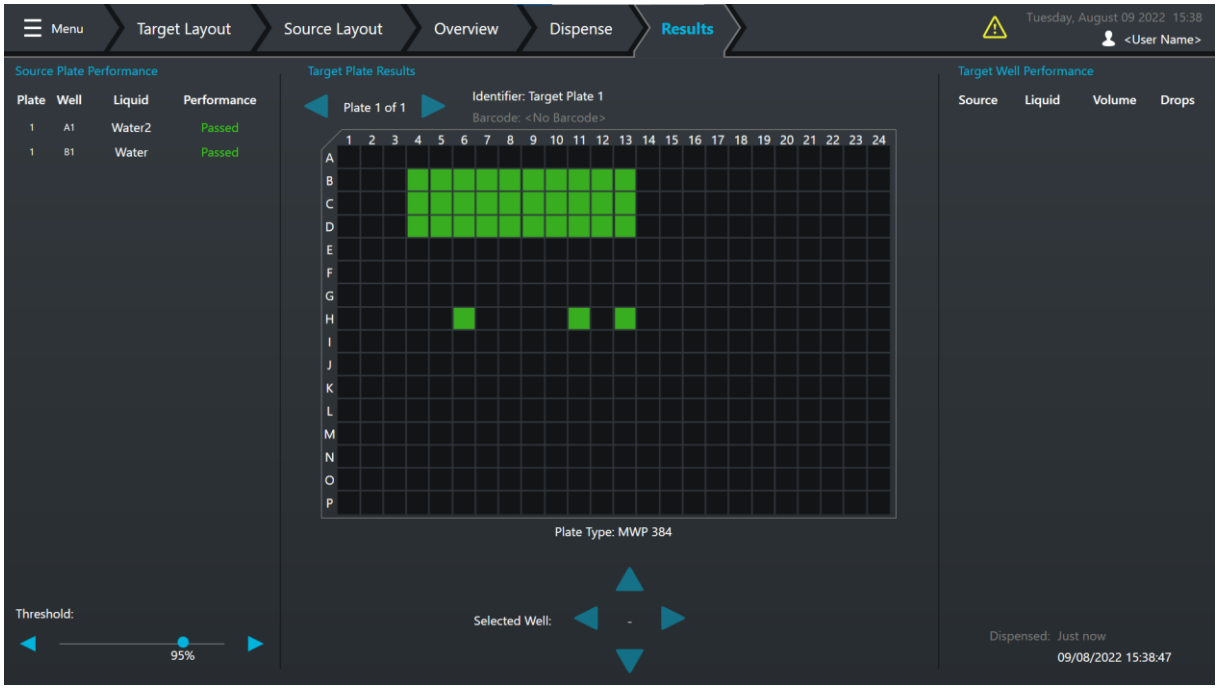


Figure 25: Results of a dispense run.

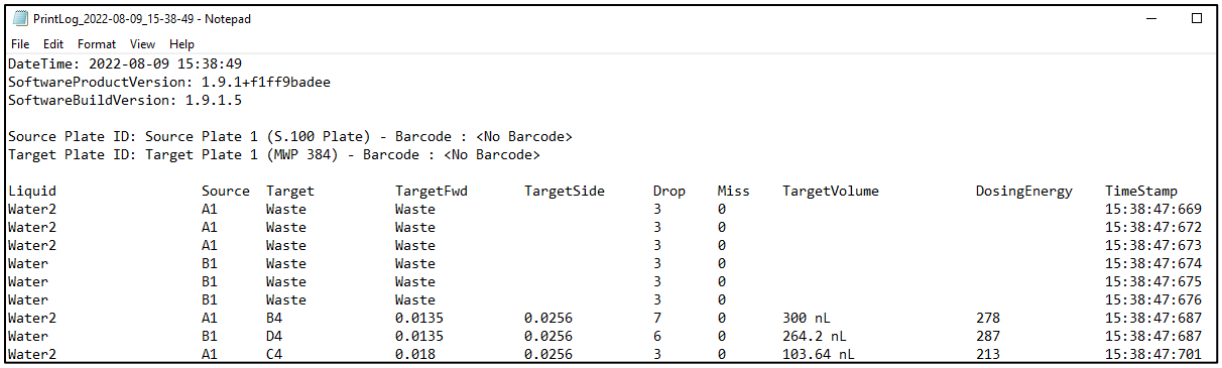
If less volume than aimed is allowed, a **threshold** for the result tab can be adjusted to a tolerable percentage. As long as the number of dispensed droplets is **above** the threshold, these target wells are shown in **green** color. All droplet numbers **below** the threshold are shown in **red**. **Yellow** color indicates passed wells but being on the **limit** of the set threshold.

For further questions on the results tab, please contact support@dispendix.com.

The **results** of the dispensing run are automatically **saved as a "PrintLog.txt" file**. To review the **graphical output** of the results, printlogs can be opened in the *I.DOT Assay Studio* software.

3. PrintLog

The printlog summarizes the results of a dispensing run within a text file.



PrintLog_2022-08-09_15-38-49 - Notepad

File Edit Format View Help

```
DateTime: 2022-08-09 15:38:49
SoftwareProductVersion: 1.9.1+f1ff9badee
SoftwareBuildVersion: 1.9.1.5

Source Plate ID: Source Plate 1 (S.100 Plate) - Barcode : <No Barcode>
Target Plate ID: Target Plate 1 (MWP 384) - Barcode : <No Barcode>
```

Liquid	Source	Target	TargetFwd	TargetSide	Drop	Miss	TargetVolume	DosingEnergy	TimeStamp
Water2	A1	Waste	Waste		3	0			15:38:47:669
Water2	A1	Waste	Waste		3	0			15:38:47:672
Water2	A1	Waste	Waste		3	0			15:38:47:673
Water	B1	Waste	Waste		3	0			15:38:47:674
Water	B1	Waste	Waste		3	0			15:38:47:675
Water	B1	Waste	Waste		3	0			15:38:47:676
Water2	A1	B4	0.0135	0.0256	7	0	300 nL	278	15:38:47:687
Water	B1	D4	0.0135	0.0256	6	0	264.2 nL	287	15:38:47:687
Water2	A1	C4	0.018	0.0256	3	0	103.64 nL	213	15:38:47:701

Figure 26: PrintLog.txt file with drop detection information for each dispensing step.

Table 1: Description of items saved in the printlog.

Content	Meaning
DateTime	Date and time of performed dispensing run
Software Product Version	Current Software version of the device
Software Build Version	Software release date
Source Plate ID	Name of used source plate and source plate type
Target Plate ID	Name of used target plate and target plate type
Liquid	Name of liquid used for the respective dispensing step
Source	Source well position for the respective dispensing step
Target	Target well position for the respective dispensing step
TargetFwd/Side	Target position coordinates on the target plate in mm
Drop	Total number of droplets counted for a source-target constellation
Miss	Total number of missing droplets for a source-target constellation
TargetVolume	Protocol setup of the target volume for the respective dispensing step
DosingEnergy	Calculated dosing energy for the respective dispensing step
TimeStamp	Actual time, when the respective dispense step was performed

All *PrintLogs* are automatically labelled with **date and time** of the dispensing run and are saved in the **subfolder "PrintLogs"** of the *I.DOT Assay Studio* directory.

4. Comma-Separated Values (CSV) File

The CSV function can be utilized for **importing** and **exporting** I.DOT dispensing protocols.

Each protocol can be created using either the *I.DOT Assay Studio* software or the CSV function. When opening a CSV file in the I.DOT Assay Studio there is the option to open it either in the Read-only Mode, where the protocol is not editable in the software, or in the Edit Mode (Figure 27).

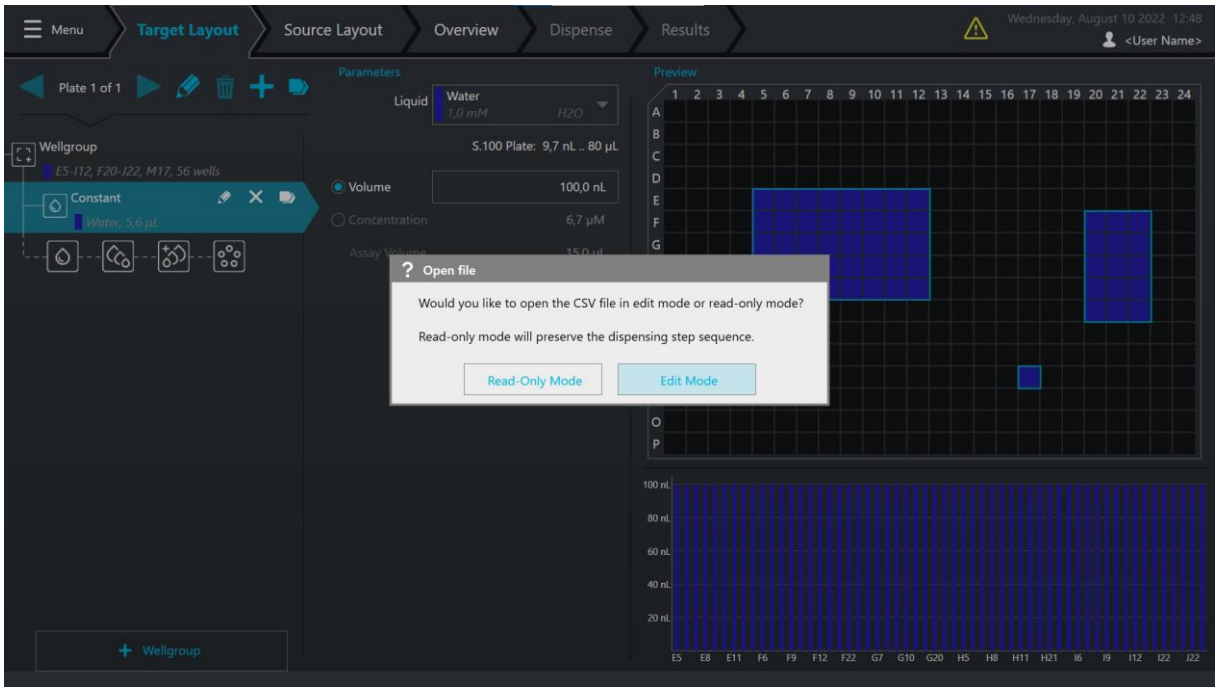


Figure 27: Open a CSV-file.

The CSV file for the use on the I.DOT device consists of a **header** and a **task section**.

	A	B	C	D	E	F	G	H
1	<Protocol Na	1.9.1.5	<User Name>	08/09/2022	15:47			
2	S.100 Plate	Source Plate 1		8,00E-05	MWP 384	Target Plate 1		Waste Tube
3	DispenseToV	DispenseToV	DispenseToV	UseDeionisat	Optimization	WasteErrorH	SaveLiquids=Ask	
4	Source Well	Target Well	Volume [uL]	Liquid Name				
5	A1	B4	3,00E-01	Water2				
6	B1	D4	2,64E-01	Water				
7	A1	C4	1,04E-01	Water2				
8	A1	D4	3,58E-02	Water2				
9	A1	B5	2,70E-01	Water2				
10	B1	D5	2,68E-01	Water				

Header

Task

Figure 28: CSV format of an I.DOT protocol.

4.1. Header Structure

The structure and content of the header section is declared as follows:

Table 2: Description of header content of the CSV file (Part 1 of 2).

Cell number/Content	Meaning
A1	Name of the protocol
B1	*I.DOT Assay Studio* software version
C1	User of the protocol
D1	Date of protocol setup
E1	Time of protocol setup
A2	Source plate type
B2	Name of the source plate
D2	Maximum fill volume
E2	Target plate type
F2	Name of the target plate

Table 3: Description of header content of the CSV file (Part 2 of 2).

Cell number/Content	Meaning
H2	Position of the waste well on the target carrier
DispenseToWaste	Enable/disable priming before dispensing (=True/False)
DispenseToWasteCycles	Number of priming cycles for each source well (=1/2/3)
DispenseToWasteVolume	Dispensing volume for each priming cycle (=5e-8/…/1e-6)
UseDeionization	Enable/disable deionization of source and target plates (=True/False)
OptimizationLevel	Used protocol optimization process to reduce total dispensing time (=NoOptimization/Reorder/ReorderAndParalell)
WasteErrorHandling	Checkpoint for the dispensing run if no droplets are detected during priming (=Ask/Abort/Continue)
Save Liquids	Checkpoint for Liquid Library handling (=Ask/Never)
Source Well	Enter position of source well for respective dispensing step
Target Well	Enter position of target well for respective dispensing step
Volume [µL]	Enter volume in microliter for respective dispensing step

Liquid	Enter exact name of liquid for respective dispensing step

The structure and content of the header section is **crucial for proper import** of .csv files into the *I.DOT Assay Studio* software. The **4th line** of the Header should **never** be changed!

4.2. Task Section

All **dispensing steps** of the dispensing run are defined within the **task section**. As intended by the header, each dispensing step has a defined source well position, target well position, a desired volume in microliter and the **exact** name of the used liquid from the liquid library. Each dispensing step should be added row-wise to the CSV list until the protocol is completed.

5. Advanced User Settings

These settings are not necessarily required for operating the I.DOT but are useful to adjust the dispensing runs for individual needs.

5.1. Liquid and Liquid Class Library

All data on different liquids and their dispensing parameters are saved in the different libraries.

5.1.1. Liquid Library

The *Liquid Library* displays all liquids that have been used for protocols. Liquids are shown with their **stock concentration** and chosen liquid class. Liquids can be deleted or added.

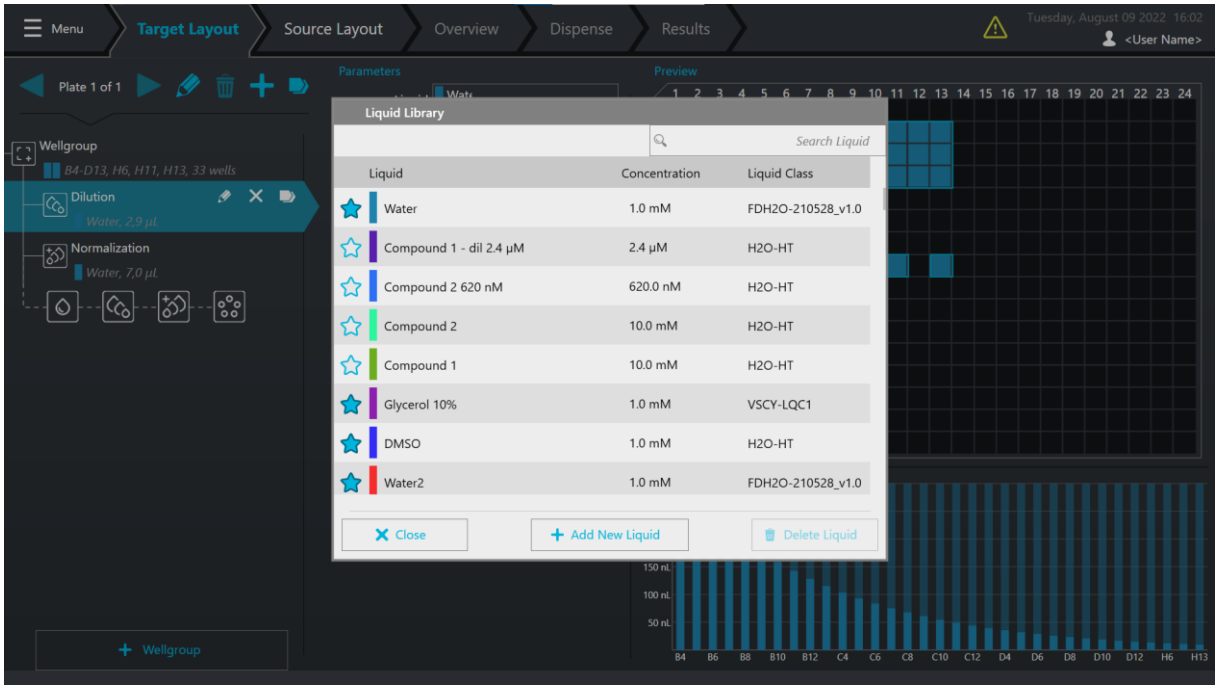


Figure 29: Liquid list in the Liquid Library.

By clicking on one liquid and selecting the button, editing this liquid is enabled (Figure 30).

The information about the **compatibility** between a **liquid** and the I.DOT **source plates** is saved for each liquid respectively.

For more information about the different source well plates, check chapter 5.1.2 Liquid Class Library.

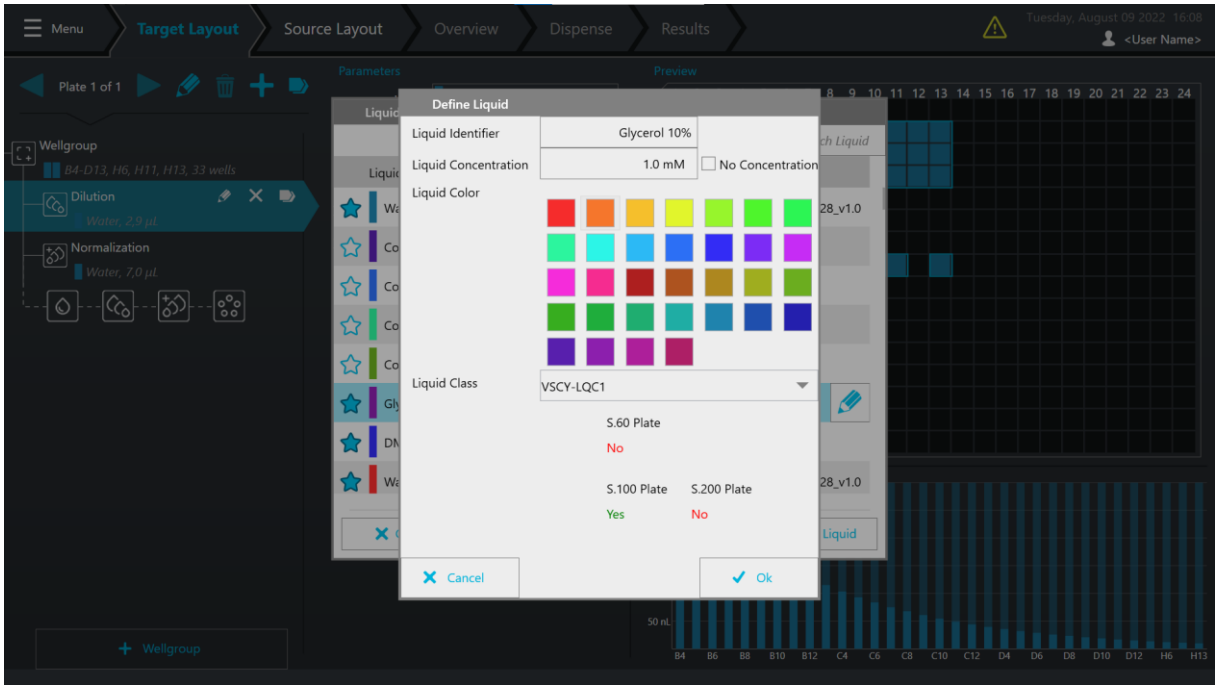


Figure 30: Define Liquid window.

☞	Assign **favorite liquids** by selecting the blue star in front of each liquid. Favorite liquids will show up in the drop-down menu, when choosing a liquid for any dispensing option while creating a protocol.

5.1.2. Liquid Class Library

The Liquid Class Library contains the **dispensing parameters** for **different liquid types** like aqueous liquids, DMSO, glycerol, solvents, etc., for the following I.DOT source plate types:

- S.60 Plate
- S.100 Plate
- S.200 Plate

The number represents the **well orifice size** (60, 100 or 200 µm).

New liquid classes can be added either by **importing** provided **I.DOT XML Liquid Class files** (*.libx) or creating new liquid classes.

> Liquid classes cannot be deleted without enabling the advanced settings. For more information on the advanced settings, see chapter 5.4. About advanced device settings.

5.2. Creating a New Liquid Class

Select **Add New Liquid Class** and enter a name for the new liquid class. Choose the desired source well type and select **Define**.

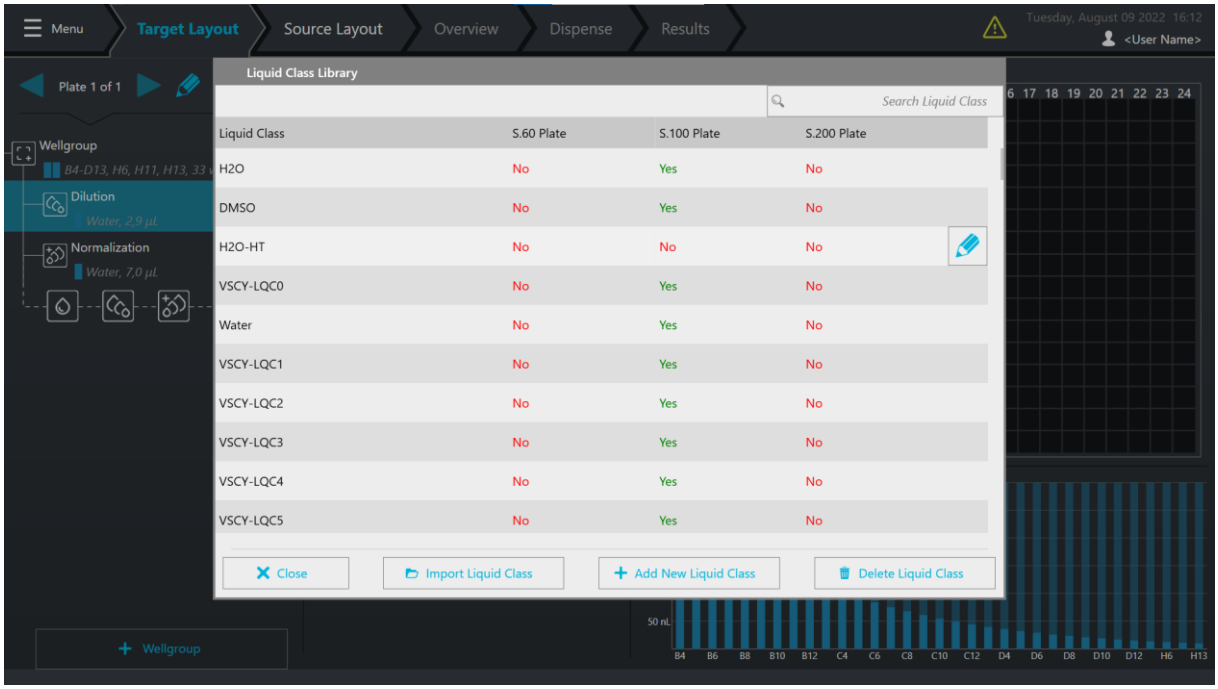


Figure 31: Liquid Class Library.

Choose one of the following options to define a new liquid class:

- Copy from existing: Dispensing parameters are copied from an existing liquid class, which can be edited thereafter.
- Measure: Automated creation of the parameters. Detailed description in section *5.2.1 Automated Creation of a new Liquid Class*
- Manually: Enter parameters manually. Detailed description in section 5.2.2 *Manual Creation of a Liquid Class*

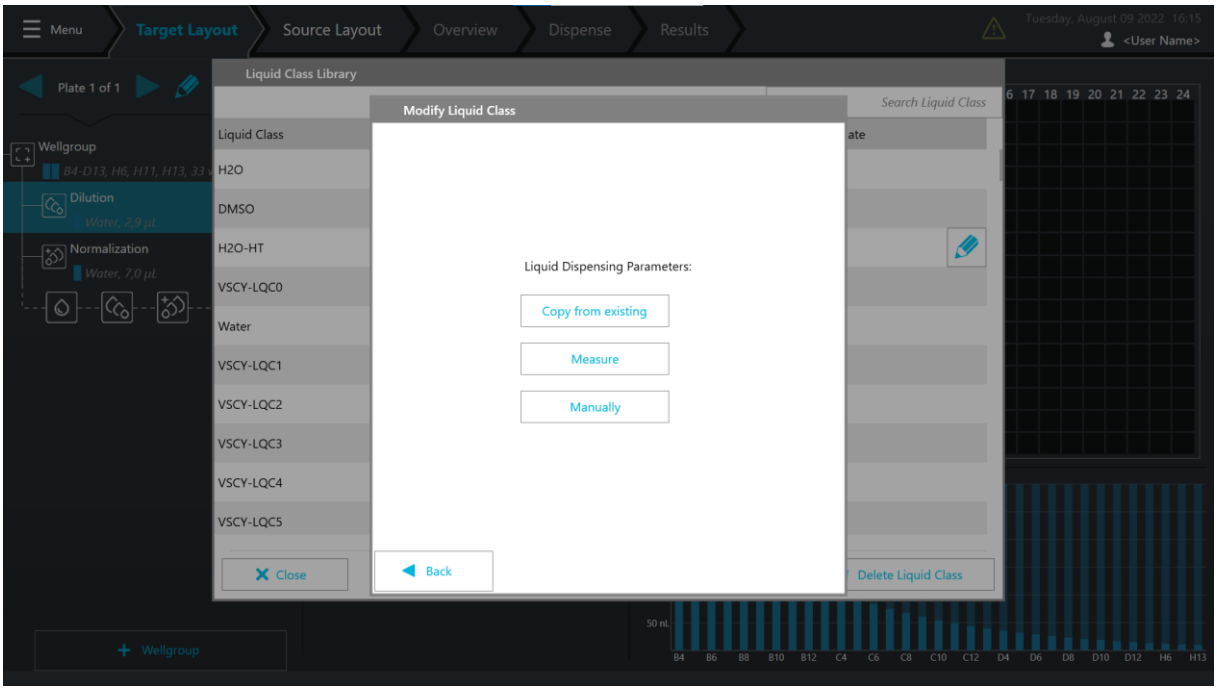


Figure 32: Adding a new liquid class: Choose, how to create the new parameters.

5.2.1. Automated Creation of a new Liquid Class

A *Liquid Class Wizard* guides through all required steps to easily measure and set up individual liquid classes.

Following steps have to be performed to measure a liquid class:

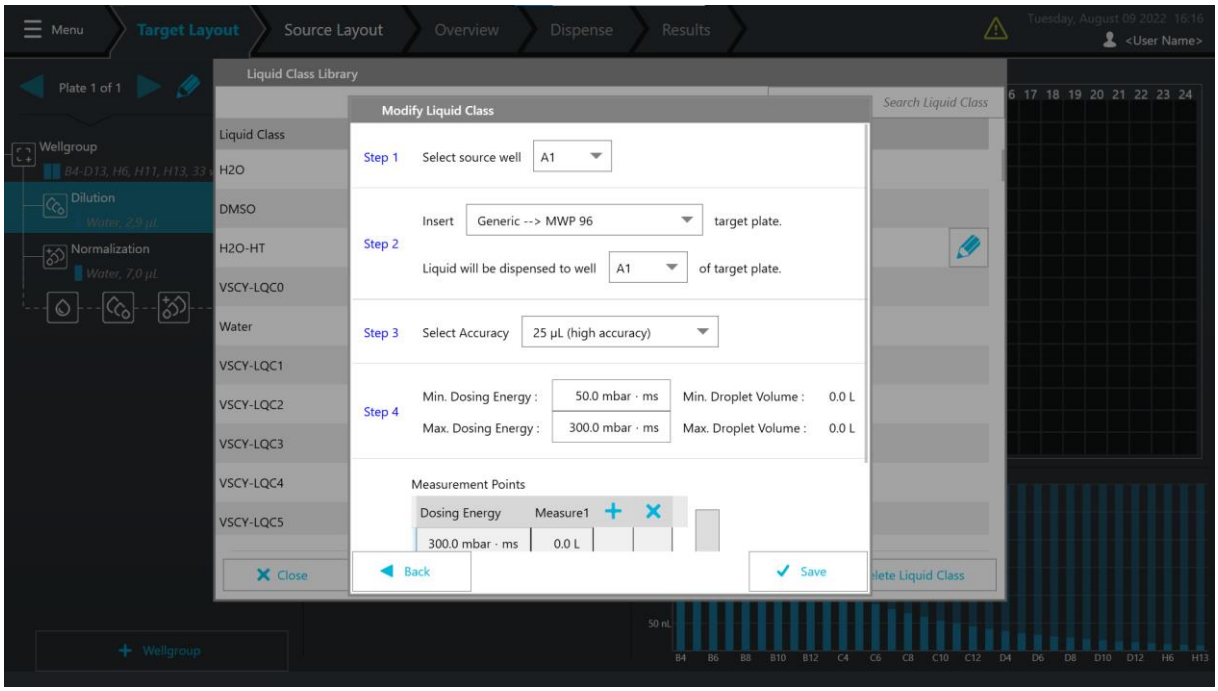


Figure 33: Liquid Class Wizard

- Step 1: Define position of source well for liquid class calibration.
- Step 2: Select target plate format and position for waste disposal.
- Step 3: Select the liquid volume that shall be filled into the source well.
- Step 4 (optional): Edit the minimum and maximum dosing energy for the liquid class.
- Step 5: Define, which pressure settings shall be used for the calibration and how many measurement points per pressure setting shall be generated. Calibration at more than one pressure setting is possible.

To start the measurement, select one row of the table, click on Measure selected row and follow the instructions of the software.

After the measurement is finished, the software calculates the liquid class from the generated data. To show the resulting graph, click on the arrow next to the table. The graph can be intersected with [0,0] by enabling the respective checkbox (Figure 34).

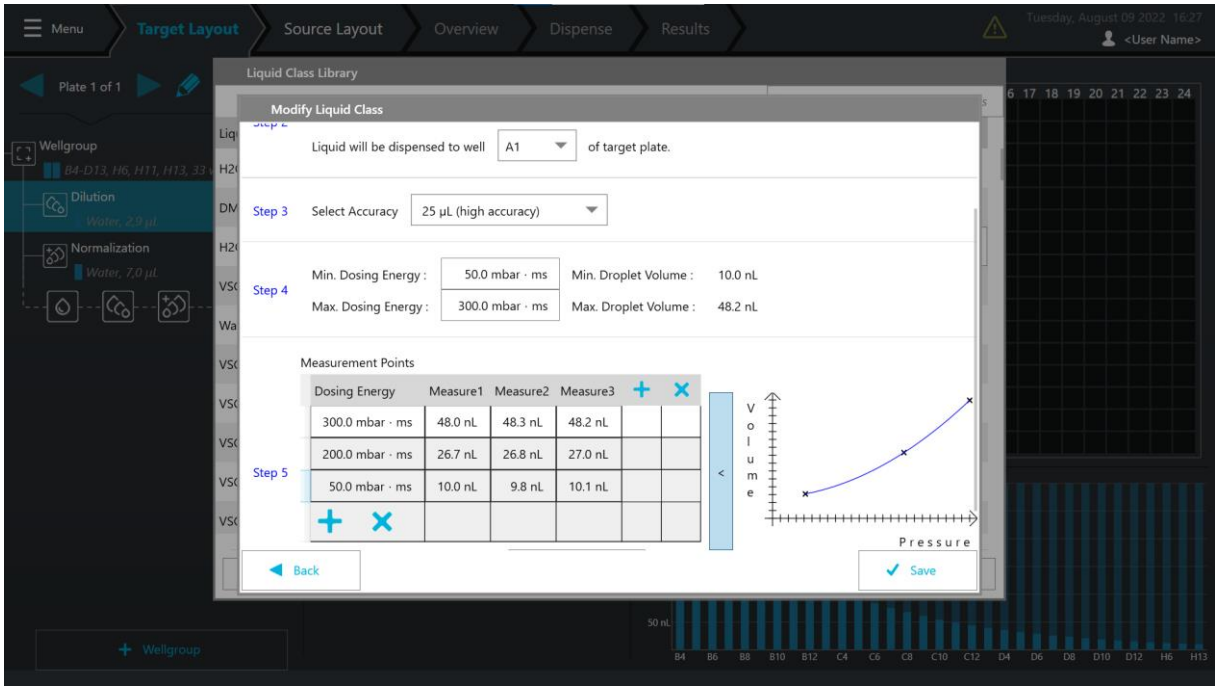


Figure 34: Measured values and plotted graph of the liquid class in the Liquid Class Wizard

.

Select **Save** and **Finish** to save the liquid class in the Liquid Class Library.

For more information on how to adjust individual liquid classes properly, please contact support@dispendix.com.

Using a **calibrated pipette** to fill the well for calibration is highly recommended. The higher the filling volume of step 3, the more precise the result will be. Measurement of **triplicates** is recommended to confirm that the measured droplet volumes are stable.

As soon as the liquid class is saved, it cannot be edited anymore.

5.2.2. Manual Creation of a Liquid Class

According to the specific correlation of dosing energy and generated droplet volume, a liquid class can be defined as a polynomial equation. By modification of the individual parameters, the

liquid class can be adjusted manually. Adjust the **parameter values** for “M”, “N”, “Min” and “Max” (Figure 35).

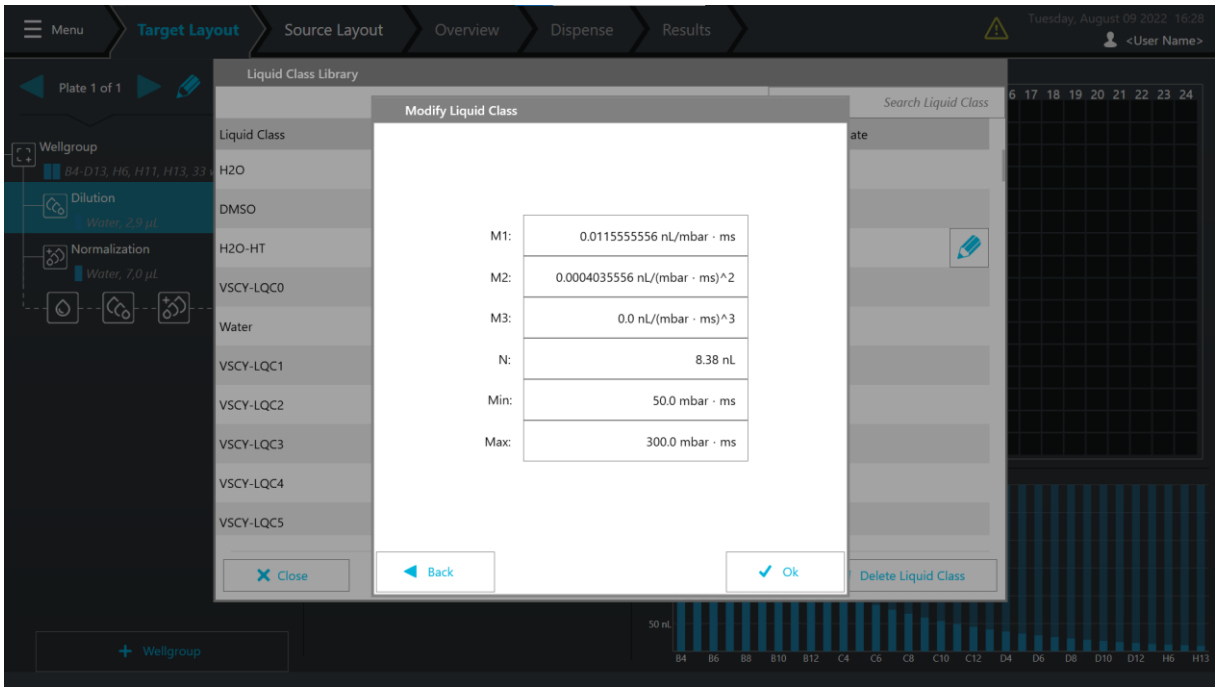


Figure 35: Liquid class parameters and values.

- **M (Slopes):** Defines the generated droplet volume in nanoliter (nL) upon application of 1 mbar*ms during the pressure pulse.
- **N (Offset):** Default 0 nL. This value describes the intersection of graph with the Y-axis.
- **Min (Minimum dosing energy):** Default 50 mbar*ms. Defines the lower pressure limit to be applied for the respective liquid class.
- **Max (Maximum dosing energy):** Default 300 mbar*ms. Defines the maximum pressure limit to be applied for the respective liquid class.

Select **Save** and **Finish** to save the liquid class in the Liquid Class Library.

The unit **mbar*ms** describes the relationship of pressure and mechanical valve opening time.

5.3. About Plate Format Library

The plate format library contains target plate formats, which are used with the I.DOT. Standard **SBS format** plates like 96-, 384- and 1536-well plates are saved by **default**. New and **individual target formats can be created** in the software using the plate format editor (Figure 36).

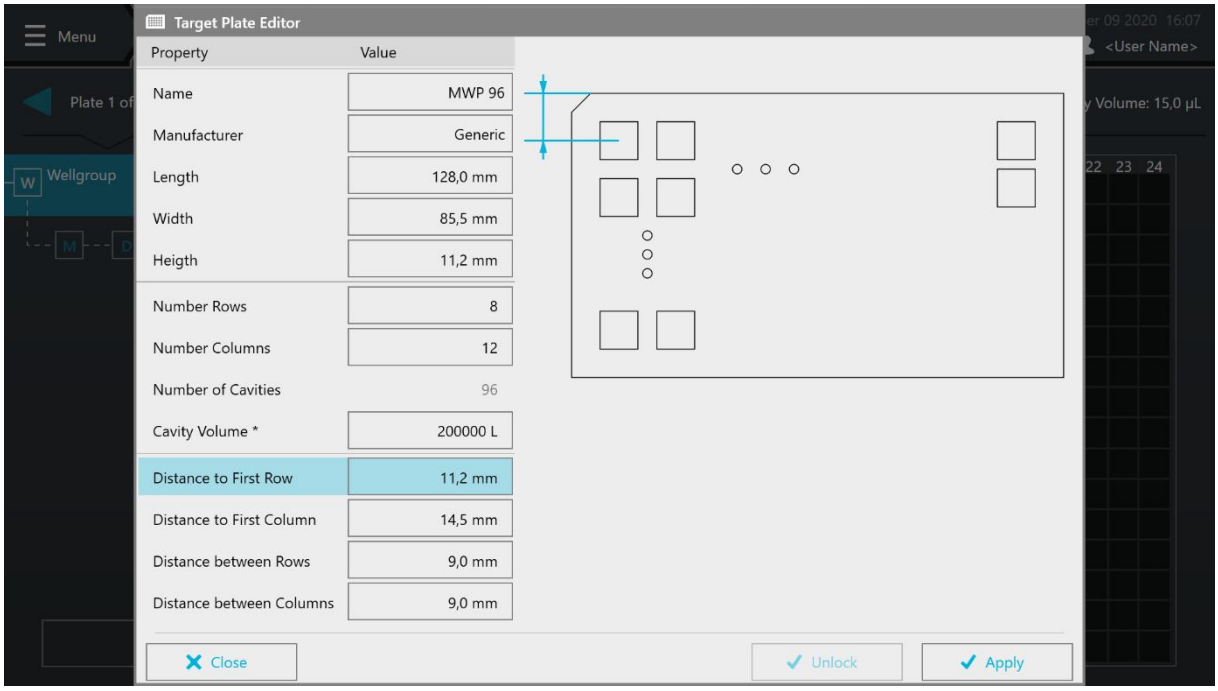


Figure 36: Target Plate Editor window.

Following items can be edited:

- **Name**: Insert plate format name.
- Optional: Edit plate **manufacturer´s name.**
- **Length/Width/Heigth**: Enter the outer dimension of respective plate format.
- **Number of Rows/Columns**: Enter value of respective plate format.
- Optional**: Cavity Volume**: Enter max. filling volume of plate format.
- **Distance to First Row/Columns**: Enter value of distance shown in editor.
- **Distance between Row/Columns**: Enter value of distance shown in editor.

Cavity volume does not have any impact on the plate format itself. Exceeding volume is not counted as error since this information is only relevant for the user.

5.4. About Advanced Device Settings

By applying the **password "Dispendix"**, advanced settings are enabled (Figure 37, red circle). Within the advanced settings position and dispense head settings can be addressed.

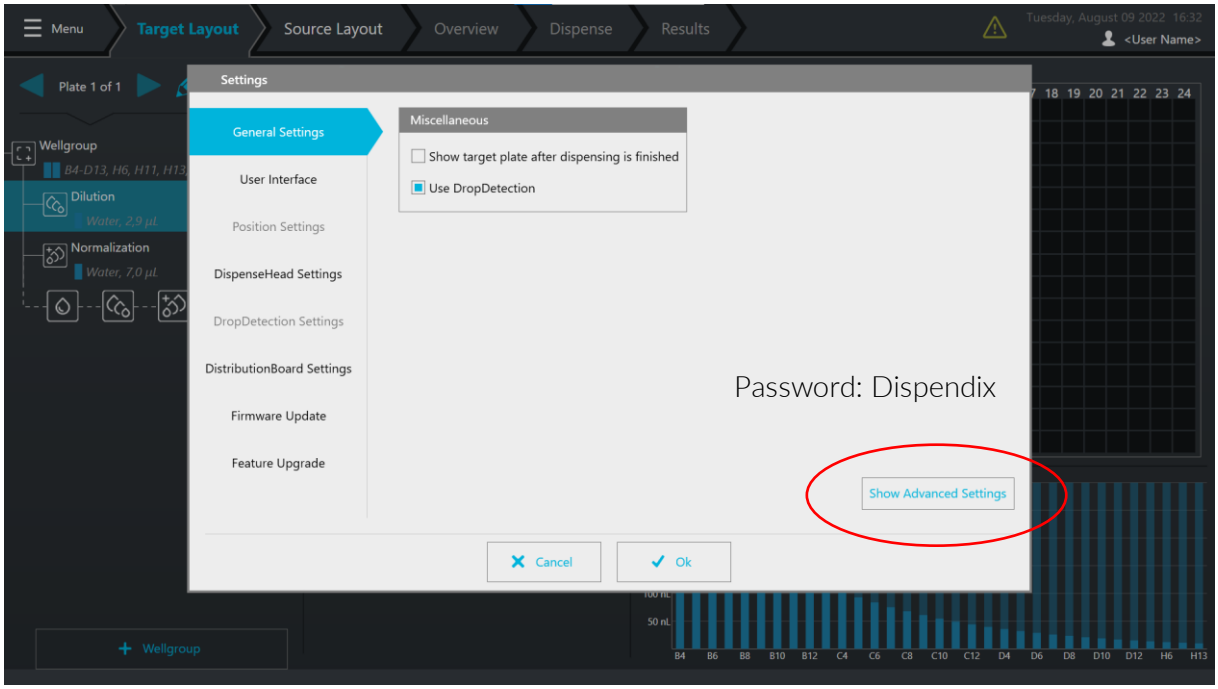


Figure 37: Device Settings screen – Show Advanced Settings (red circled).

5.4.1. Reset Liquid Classes

In case the original liquid classes provided by Dispendix are deleted or modified and shall be restored, click on the button **Reset Liquid Classes** in the general settings window (Figure 38, red circle).

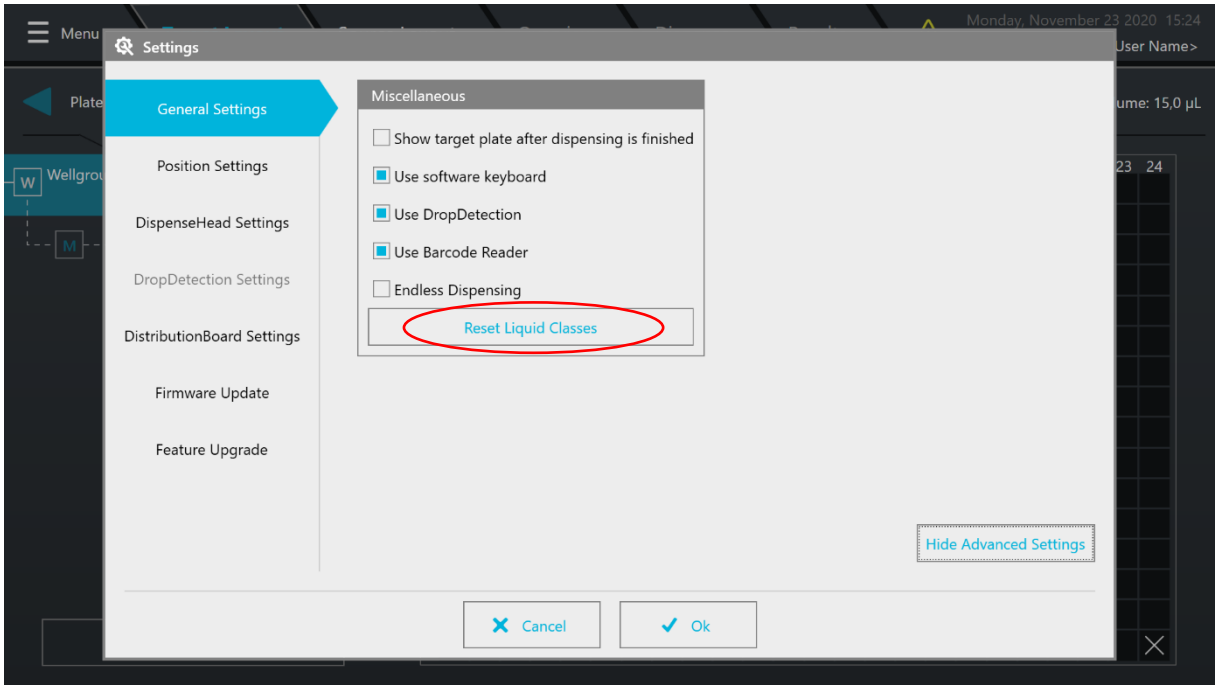


Figure 38: Restoring original liquid classes – Reset Liquid Classes (red circled).

Reset liquid classes does not delete individually created liquid classes!