IMAPlate Q&A

Q1. What is the actual volume of solutions that the IMAPlate 5RC96 will take up by using capillary force loading method?

A: The geometric volume of each reaction chamber of the IMAPlate 5RC96 is 5µl. The actual volume of solution it takes up by using capillary force loading method is expected around 5.5µl because the IMAPlate 5RC96 is designed to use this addition volume to obtain a better surface shape between liquid-air interfaces for the reader. The actual volume may have a slight change due to the surface tension of different liquids (as also seen in other liquid transfer device such as pipette). For example, the volume for taking up water is 5.6µl with CV less than 2% (from 10 IMAPlate 5RC96). Therefore, it is recommended to perform a calibration when the IMAPlate 5RC96 is used as a liquid transfer device. For other applications such as micro-cuvette array or solid phase assay the difference between the geometric volume and the actual volume will have **no influence** on the result as long as the standards and samples are loaded by using the same method.

Q2. How precise of the IMAPlate 5RC96 to take up solutions by using capillary force loading method?

A: The CV% of taking up solutions (e.g. PBS or PBS-0.05%Tween20) among the reaction chambers in the whole IMAPlate 5RC96 is less than 10% (usually around 5%) in the dye dilution test.

Q3. What is the required volume of solution for each well of 96-well plate in order to fully load the IMAPlate 5RC96 by capillary force loading method?

A: Minimum and maximum volume of the solution in the well of the source plate (96-well plate) should be pre-determined as different brand plates may be different. The minimum volume shall enable the very end of the reaction chamber of the IMAPlate to have the contact with the solution after putting back the fully loaded IMAPlate into the source plate and the maximum volume shall not allow the level of the solution above 3mm of the very end of the reaction chamber once the empty IMAPlate is put on the source plate. For instance, the addition of 20 – 50µl of solution in Nunc V- or U-bottomed 96-well plate or 80 – 150µl in Nunc F-bottomed 96-well plate will give good results.

Q4. Why should I move the IMAPlate down-and-up during the capillary force loading method?

A: As the IMAPlate is very hydrophobic, the hydrophilic solution may not automatically flow into the reaction chamber. A down-and-up procedure, in which the IMAPlate is placed on the source plate and then is lifted up till the very end of the reaction chamber leaves the surface of the solution, is required in order to fill up the reaction chamber. The times of this procedure may differ due to the speed and the force applied. Usually, 10-time of the down-and-up procedure will be enough for a trained person to fully fill up the reaction chamber. Please be aware that to lift the IMAPlate from the

source plate **very slowly** for **the last time** to eliminate the formation of any droplets outside the reaction chamber.

Q5. How can I load IMAPlate with a pipette for small volume reaction?

A: You can pipette **up to 6µl** of solution to overload the reaction chamber for small volume reaction from either top or bottom openings. In both cases, make sure the pipette tip end is within the openings of the reaction chamber before dispensing the solution. The solution will flow into the reaction chamber and remain inside by capillary force. **Only can the pipette button be released when the tip end has no contact with the solution in the reaction chamber.** Otherwise the solution will flow back into the tip.

Q6. Can I load IMAPlate several times with a pipette for small volume reaction?

A: Yes, you can pipette IMAPlate **several times**. But the **total** volume should not excess 6µl. In order to not make bubbles, the tip end should be in the solution, which is already inside the reaction chamber, before dispensing the solution.

Q7. Can I pipette 15 µl or more solution to IMAPlate for macro volume reaction and analysis.

A: Yes, you may pipette 15 to 50µl of solution from top opening to the upper compartment of IMAPlate for analysis or reactions. The maximum volume should be determined because the amount of solution the IMAPlate can hold may vary due to the different composition of solutions.

Q8. How can I measure DNA/RNA and protein absorbance by using IMAPlate 5RC96 as a micro-UV-cuvette array? How about the CV%?

A: You should always use two-wavelength measurement method in order to get true absorbance (See product insert). It is recommended to use the same liquid loading method for standards and samples in order to get accurate results. The CV% is usually around 5% for a sample in the plate by using capillary force loading method.

Q9. Can I use the white IMAPlate 5RC96 for fluorescence measurement?

A: Yes and the background will be high. Use black IMAPlate for fluorescence measurement.

Q10. Can I reduce assay time by using the IMAPlate 5RC96 to replace 96-well plate?

A: Of course, when the reaction is heterogeneous and uses the wall of the reaction chamber as a solid phase. A satisfied result can be achieved by coating capture antibody only for 30 to 60min at room temperature as well as by reducing half or more of the time for antibody and antigen reaction with a standard ELISA protocol for 96-well plate.

Q11. How can I stop the TMB color reaction in IMAPlate 5RC96 ELISA?

A: There are several strategies to stop the reaction. 1) You can pipette 0.5µl of concentrated acidic solution to each reaction chamber. 2) First you use another IMAPlate to **spot** concentrated acidic solution on a flat surface such as the cover of microwell plate. Then you make the bottom opening of the reaction chamber to contact with the correspondent droplet of acidic solution for a certain time. The acidic solution will stop the color reaction **simultaneously**. 3) You can add 15µl or less of concentrated acidic solution to a 96-well plate with V shape bottom, put the IMAPlate on and make sure the bottom opening of the reaction chamber to contact with the acidic solution for a certain time to allow the acidic solution to exchange with the solution in the reaction chamber. The reaction will stop **simultaneously**. 4) You can use NCL TMB substrate solution. The reaction can **simultaneously** be stopped by using a filter paper to emptying the reaction chamber at the same time. The NCL TMB substrate solution is especially designed for high-throughput and provides users the maximum flexibility. It can decrease the background to the minimum.

Q12. How can I prevent the evaporation of the solution in the IMAPlate 5RC96 during a longer incubation?

A: You can put the IMAPlate 5RC96 on the reader adapter that is sitting on a wet flat filter paper, which is on a solid flat surface (e.g. lab table, 96-well plate lid and so on). And then cover the IMAPlate 5RC96 by another wet filter paper on the top.

Q13. Can I use the IMAPlate 5RC96 for add-and-mix homogeneous assay and what is the advantage?

A: Yes, you can first seal the bottom openings with a sheet of parafilm by pushing the IMAPlate down to a 96-well plate covered with a suitable sized parafilm. Then you pipette assay components one by one to the upper compartment and mix thoroughly. Remove the IMAPlate from the 96-well plate and peel off the parafilm. Gently tap the IMAPlate to ensure the assay solution flowing to the reaction chamber and finally place on the IMAPlate adaptor for the measurement. The total volume of the assay should not be below 15 µl for this macro volume reaction procedure.
The advantage is 1) increase the detection sensitivity, 2) reduce the assay volume and 3) easy to handle.