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Direct Reading Grid
User Manual

Direct Reading Grid User Manual

Revision 1, April 2023

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Description

This user manual provides technical and usage information to users of the Direct Reading Grid.

Components	5 Units of Direct Reading Grid
Storage	Store grids in a cool and dry place. When unused, always keep grids enclosed in packaging.
Precaution	Ensure grid is dry before use. Do not use if damage is visible. Do not autoclave or soak the unit in ethanol. Do not wipe or scratch the underside of the DRG.

The Direct Reading Grid is for use with the Laminar Wash™ 96-well plate only. It is not suitable for use with conventional round-bottom plates or other Curiox patented plates.

Background Information

The Direct Reading Grid (DRG) is a disposable accessory that sits on top of the Laminar Wash™ (LW) 96-well plate (96-DC-CL-05 or 96-DC-CL-05B), forming physical walls around each well. Its purpose is to increase the total holding volume of the plate, thereby enabling direct acquisition of samples for flow cytometry-based applications. The DRG is available in two configurations:

- Automation friendly DRG (DC-GR02-96-A) – no Gripper added
- Manual compatible DRG (DC-GR02-96-M) – Gripper added on either side

***Disclaimer*:** This product is intended for one-time use.

Application

The DRG is meant for direct acquisition on flow cytometers. A list of validated cytometers for use with the DRG and tested parameter settings can be found below in Appendix. The acquisition parameters should be used as directions and may require optimization with your specific application and cytometer. For use on cytometers other than those validated, please reach out to your regional FAS for assistance.

The DRG is not prescribed for reagent incubation, or for use inside the Curiox Laminar Wash™ station.

Technical Specifications

Part Numbers

GR-LW-96-M. Direct Reading Grid

GR-LW-96-A. Direct Reading Grid for Laminar Wash™ AUTO1000 station (AUTO-1000-96-01)

Definitions

Static Holding Volume. Holding volume of fluid in a LW plate well with the DRG without any form of disturbance.

Rough Handling Capability. Simulated rough handling of the LW plate with DRG where the content in the well does not spill or leak out of the confinement.

Quick Dispensing Leakage Resistance. Ability of the DRG to resist leakage as fluid is dispensed into the well at high flow rate.

Rotary Stirring. Linear velocity of the rotational motion of a mixing probe performing a mixing action.

Pipette Mixing. Quick dispensing and aspiration of fluid content in a well for the purpose of inducing a mixing action.

Orbital Acceleration. Acceleration experienced by shaking or orbital motion.

Product Information

Fluidics

Description	Specification
Operating temperature range	4-37°C
Maximum static holding volume per well	300 µL of 10% FBS/PBS for <24 hr 200 µL of 1% BSA/PBS for <24 hr
Vortexing capability	Vortexing speed 1650 rpm Vortexing span 1.0 mm Vortexing time max 30 sec ≤250 µL of 10% FBS/PBS
Quick dispensing leakage resistance	≤100 µL/s of 10% FBS/PBS With pipette tips directed near center of well bottom
Rough handling capability	With 300 µL of 10% FBS/PBS: Orbital speed 200 rpm, 10° tilt on automated rocker or 45° static tilt With 200 µL of 1% BSA/PBS: 45° static tilt

Compatibility with Curiox Sponsored Products

The DRG is compatible for use with the following Curiox sponsored products:

- Laminar Wash™ 96-well plate with lid (96-DC-CL-05 or 96-DC-CL-05B)
- Laminar Wash™ AUTO1000 station (AUTO-1000-96-01)
- Big Bear vortexer (HT91-1000-1)
- Shaker (96C20-1001)

Usage Guidelines

Buffer Compatibility

The DRG is compatible with up to 250 µL of the following buffers when used within the above mentioned specifications:

- 10% or less FBS in PBS
- 0.5-1% BSA in PBS

The DRG is not validated with buffers containing surfactants, detergents, and protein concentrations of >10%. Ensure buffers are free of precipitates and contaminants. Old and contaminated buffers can possibly cause failure.

Handling

- Ensure DRG has not been tempered with, and it is clean and dry before use. Each DRG is for one-time use.
- Use reliable, straight, and tight-fitted pipette tips.
- Use unexpired LW plates that are completely dry only.
- Align A1 corner of DRG with A1 corner of LW plate (refer to Fig. 1).
- Ensure grid sits flatly on the plate. There should be no gap between the grid and plate as it can lead to merging (refer to Fig. 2).
- Avoid tilting or shaking the unit (DRG-LW plate assembly; refer to Fig. 2) at any time.
- Prior to cytometer acquisition, do not place the DRG-LW plate assembly at temperatures below 4°C.

When pipetting (refer to Fig. 2), align tips vertically or at an angle less than 15° of tilt, depress pipette plunger gently, aim at the center; do not aim at the grid-well interface. If the grids fail, do not try to aspirate out the liquid and continue to use the DRG. Discard the grid.

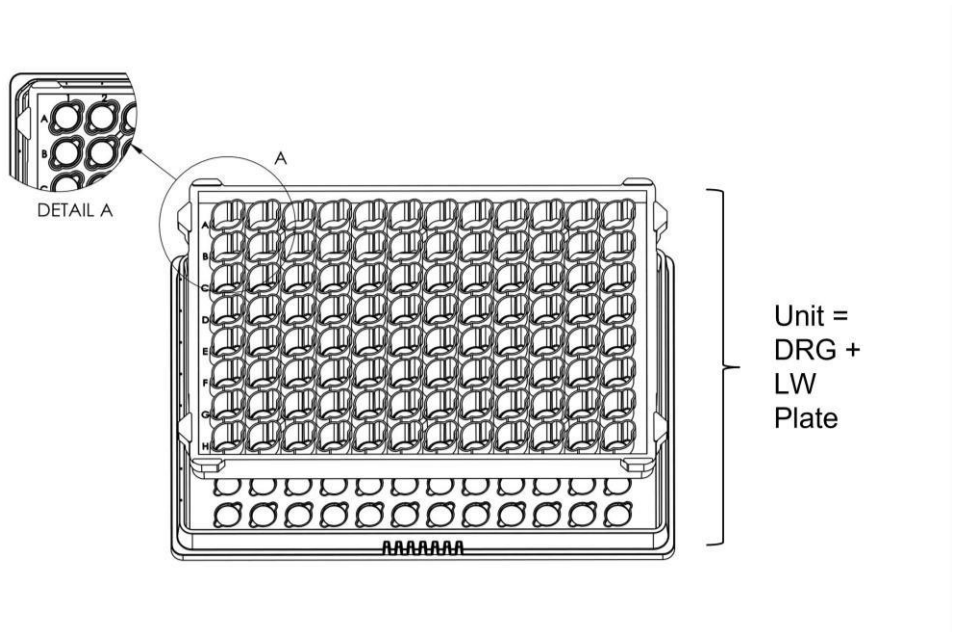

Figure 1. Image illustrating the A1 corner of the DRG (Detail A), and the DRG and LW plate assembly as a 'unit'.

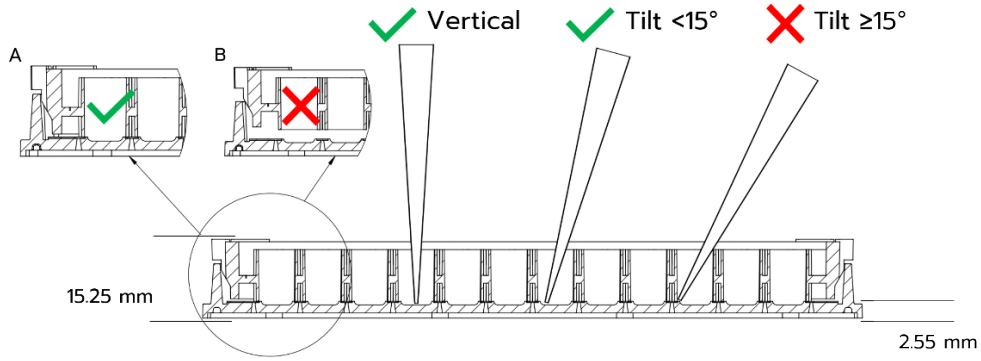

Figure 2. Image illustrating the appropriate way to align pipette tips and important measurements needed for flow cytometry acquisition. The DRG must sit flat on the LW plate with no gap in between the DRG and LW plate (A). Presence of a gap in between the DRG and LW plate when not inserted correctly (B).

Instructions for Use

A general example of a workflow is as follows:

Manual workflow (with DRG, GR-LW-96-M)

- Sample wash is completed on Laminar Wash™ station. LW plate is retrieved with 25 µL residual volume. DRG is not for use in the Laminar Wash™ station. Do not insert DRG in the Laminar Wash™ station.
- Place dry, fresh DRG onto LW plate.
- Top up wells with acquisition buffer (up to 300 µL total volume).
- Vortex on Curiox-specified shaker for 10 seconds to ensure cell resuspension.
- Read directly on cytometer (use Black Spacer, PL-SP-BK-01 if necessary to adjust height).

Automation workflow (with DRG, GR-LW-96-A)

- Place DRG onto the DRG Adaptor on the allocated deck space. Set up the AUTO1000 deck with other consumables according to instructions on the AUTO1000 Graphical User Interface (GUI) software. Ensure GUI software is v3.2.12 or later. Please contact your regional Automation Specialist if GUI software is not up to date.
- Set up automation protocol.

Disclaimer

The DRG is intended for one-time use only.

Dimensions

The DRG is 124 mm in length, 82 mm in width and 12 mm in height (refer to Fig. 3).

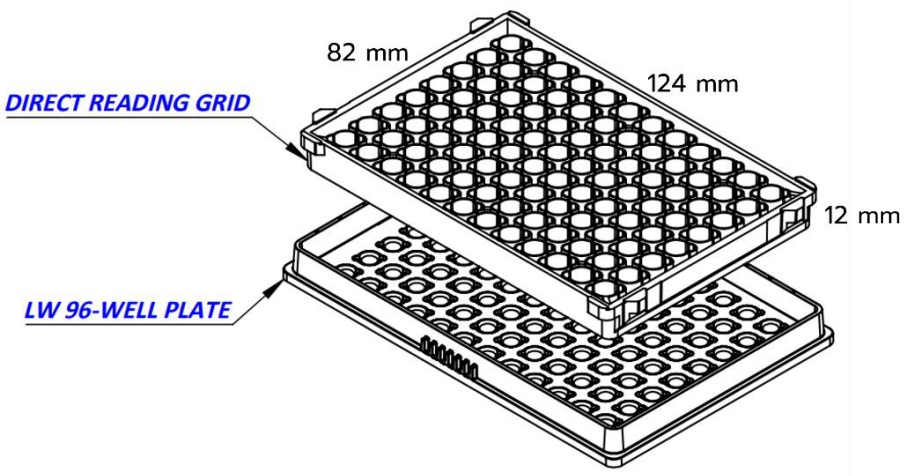

Figure 3. Image illustrating the dimensions of the DRG (124 mm x 82 mm x 12 mm).

Appendix

The table below shows the acquisition parameters tested for DRG-validated flow cytometers.

Cytometer brand and model	Acquisition volume (µL)	Mixing volume (µL)	Sample flow rate	Mixing speed	Number of mixes	Mixing time (s)	Plate type	Black spacer needed
Cytek™ Aurora (Plate loader v1.5)	250-300	NA	200 µL/s	-	NA	3	Flat-bottom plate	No
Cytek™ Aurora (Plate loader v2.0)[1]	50-300	NA	-	1200-1400 rpm	NA	2	LW plate[2]	No
BD FACSymphony™ A5 SE + HTS	150	100	-	180 µL/s	3	NA	Flat-bottom plate	Yes
BD FACSCelesta™ + HTS	120	100	1.5 µL/s	100 µL/s	5	NA	U-bottom plate	Yes
Beckman Coulter CytoFLEX LX	100	NA	Fast (60 µL/min)	-	NA	10	LW plate[2]	No
Beckman Coulter CytoFLEX S	250	-	-	-	NA	2	Flat-bottom plate	Yes
Invitrogen™ Attune™ NxT CytKick™ Max Autosampler	100	NA	100 µL/min	-	2	-	Flat-bottom plate	-