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A brief comparison of Manual and Automated dispensing of Antibodies with the I.DOT

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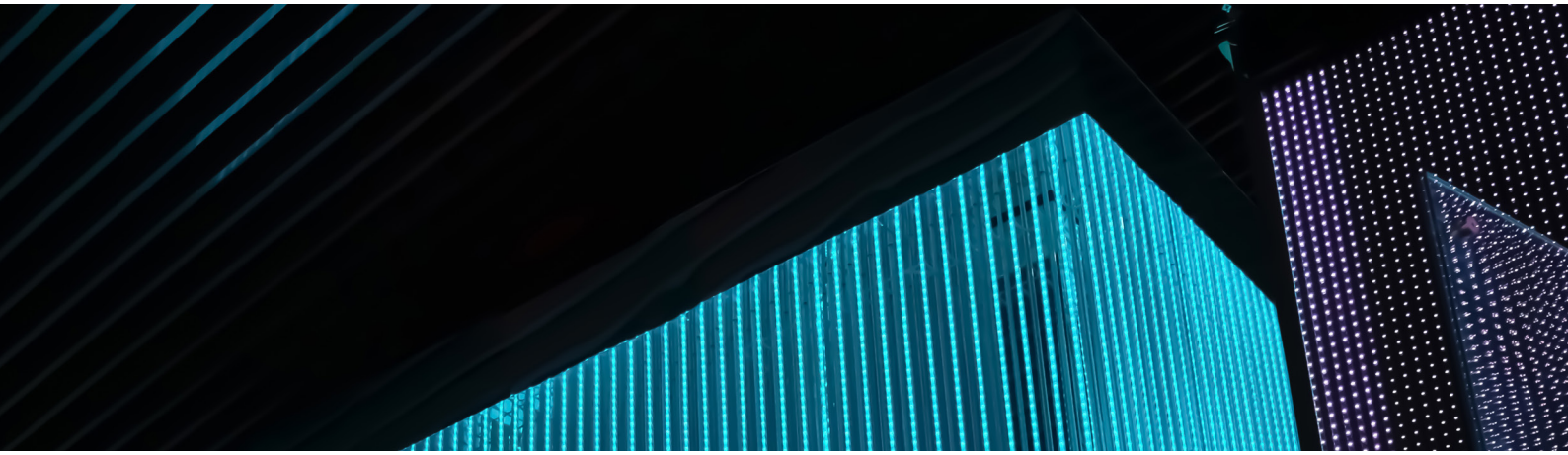

Introduction

Antibodies, also known as immunoglobulins (Ig), are glycoproteins produced by differentiated B lymphocytes known as plasma cells in response to antigen susceptibility. The antibodies can then be used in a variety of applications such as western blot (WB), immunoprecipitation (IP), immunofluorescence (IF), immunohistochemistry (IHC), chromatin immunoprecipitation (ChIP), and flow cytometry (FC).

In recent years, several types of antibodies, including primary and secondary antibodies, as well as monoclonal and polyclonal antibodies with varying specificities, have been developed. These advancements have made antibodies indispensable components in a wide range of applications in medicine, biomedical research, and diagnostics.

The broad range of antibody-related applications is undoubtedly needed in the pursuit of scientific discoveries. However, to produce reliable and reproducible results for specific experiments, antibody-based methods consume time, labor, accuracy, and money.

I.DOT and its adaptability to consumables have shown assurance in dispensing antibodies and support biomedical research and development of therapeutics. Check out the technical bulletin on antibody dispensing to help you choose the right antibody dispensing consumable.

Advantages of using the I.DOT for Antibody Dispensing

- Antibodies of various sizes can be dispensed
- Antibodies can be dispensed instantly
- Eliminates pipetting inaccuracy and dispenses precisely and accurately
- Significant reduction in human labor
- Low dead volume
- Reliable and reproducible results
- Eliminates risk of cross-contamination

Materials and methods

Antibodies with different sizes (16-160kDa) and clonality (monoclonal and polyclonal) were purchased (Table 01). Subsequently, these antibodies were diluted (1:2000) in an appropriate diluent. Next, liquid classes were created for each antibody prior to dispensing with the I.DOT.

For the measurement of concentration of antibodies in Qubit Flex Fluorometer, Standards were prepared as per Qubit Assay Kit user guide. Finally, the protein assay was performed with 20µl sample volume consist of 2 µl test sample i.e., 1:2000 antibodies and 18 µl Qubit assay working solution.

To observe if dispensing antibodies with I.DOT causes any change in the concentration of antibodies after dispensing, 2 µl test samples (antibodies) were dispensed with I.DOT and the same sample volume was pipetted in Qubit assay microtubes containing Qubit assay working solution and concentration of antibodies were determined using Qubit Flex Fluorometer. Thus, comparison was made between manual and I.DOT antibody dispensing.

S.No	Antibody	Clonality	Isotype	Protein Size in kDa	Dilution	Applications
1.	Anti-CDKN2A (p16INK4a) mouse	Monoclonal	IgG1	16.4	1:2000	IF, IHC, WB
2.	Mouse anti-GAPDH	Monoclonal	IgG1	35.9	1:2000	WB (Control)
3.	LGR5 mouse	Monoclonal	IgG1	99.8	1:2000	FC, IF, IHC, IP, WB
4.	Citrate synthetase (CS) Rabbit Polyclonal Antibody	Polyclonal	IgG	45	1:2000	IF, IHC, WB
5.	Rabbit DAPK1 Antibody (C-term)	Polyclonal	IgG	160	1:2000	IF, IHC, WB

Table 1. *Overview of Antibodies.*

Results and discussion

Dispensing antibodies with the I.DOT does not cause loss of antibody mass as indicated by a change of concentration

Following the completion of the protein assay for antibody concentration determination, the Qubit readings for antibody concentration were analyzed (Table **2** & Fig **1**)

Antibody	Manual pipetting	I.DOT dispensing
Anti-CDKN2A (P16INK4a) mouse	0.24	0.24
Mouse anti-GAPDH	0.25	0.24
LGR5 mouse	0.25	0.24
Citrate synthetase (CS) Rabbit Polyckonal Antibody	0.20	0.21
Rabbit DAPK1 Antibody	0.20	0.2
	0.24	0.24

Table 2. *Comparison of Manual and I.DOT Antibody Dispensing.*

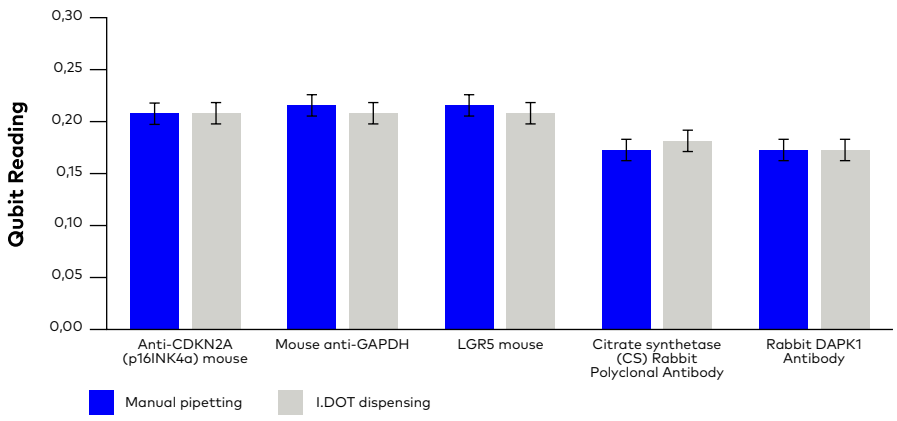

Figure 1. *Comparison of Manual and I.DOT Antibody Dispensing.*

No significant difference in the concentration of antibodies was observed between manual pipetting and I.DOT dispensing method. Hence, I.DOT does not cause any hinderance in antibody dispensing. Thus, the I.DOT can be implemented for fast and accurate dispensing of antibodies.

Conclusions

The data presented above show that using I.DOT for antibody dispensing has no effect on the concentration of antibodies dispensed. As a result, the I.DOT can be used to achieve consistent and reliable results besides dispensing different sizes of antibodies quickly and accurately.

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