

Precise and Accurate Reagent Transfers Utilizing Acoustic Droplet Ejection Offers New Opportunities in Assay Setup

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1. ABSTRACT

Acoustic droplet ejection (ADE) is a completely touchless transfer technology for liquid handling that eliminates the use of disposable tips and wash cycles. The ability to transfer from any well of the source plate to any well of the destination plate enables flexible assay setup in both 384-well and 1536-well plates. For the ADE, we used the Labcyte Echo® 555 liquid handler (Labcyte Inc.) to transfer different volumes of aqueous-based reagents from several commonly used biochemical assay kits. To measure accuracy and precision, each reagent was doped with 0.15 nM fluorescein so that the transferred volumes could be measured on an Envision reader (PerkinElmer). The volumetric precision for each reagent transferred was below 2.5% CV and the accuracy was within 10% of the expected volume. These results demonstrate that ADE provides precision and accuracy for a newly expanded range of fluid types that is comparable to established performance with DMSO. These new capabilities enable assay setup with flexible plate layout and volume reduction for reagent and consumables savings and higher throughput.

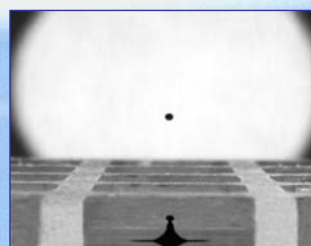
2. Material and Methods

Dispensing Precision and Accuracy

Commercially available HTS biochemical assay kits were purchased, and the reagents were reconstituted and diluted according to each kit's instructions. Each reagent was then doped with fluorescein to a final concentration of 0.15 nM, and a two-point standard curve was generated. Each reagent was loaded into a 384-well polypropylene Echo qualified source plate (Labcyte Inc.) at 40 µl per well. Using the Labcyte Echo® 555 liquid handler (Labcyte Inc.), 5 nl or 50 nl volumes were transferred from the source plate to each well of a 384-well destination plate (Greiner bio-one). 50 µl of 10 mM NaOH buffer was added, and the plates were centrifuged,

incubated for 30 minutes at room temperature, and read on 2100 EnVision plate reader (PerkinElmer). RFU values were converted to volumes against the standard curve of each reagent, provided by the kit manufacturers. Table 1 shows the reagent, the assay kit, and the obtained volumes and transfer volume CVs. Average CV was 1.31% for all reagents tested at 5 nl target volume. With the ability to dispense from one well to multiple wells, and from any well to any well, Echo liquid handlers offer excellent precision and flexibility for total assay setup.

Reagent	Kit	5nl Target Volume		50nl Target Volume	
		Ave. Actual volume (nl), n=8 wells	CV	Ave. Actual volume (nl), n=8 wells	CV
Sa-XL665	Cisbio HTRF KinEASE-STK (PN 62ST3PEB)	5.50	1.43%	53.25	1.00%
STK Ab-Cryptate	Cisbio HTRF KinEASE-STK (PN 62ST3PEB)	5.74	1.24%	56.00	0.80%
STK Substrate	Cisbio HTRF KinEASE-STK (PN 62ST3PEB)	5.24	1.27%	50.07	1.17%
anti-cAMP-Cryptate	Cisbio cAMP dynamic 2 (PN 62AM4PEB)	5.15	2.01%	51.61	1.45%
cAMP-d2-Cryptate	Cisbio cAMP dynamic 2 (PN 62AM4PEB)	5.76	2.14%	54.87	1.54%
anti-IL1B-Cryptate	Cisbio human IL1B (PN 62IL1PEB)	5.78	0.75%	58.43	0.55%
anti-IL1B-XL665	Cisbio human IL1B (PN 62IL1PEB)	5.76	0.48%	60.43	1.01%
anti-IL1B-Calibrator	Cisbio human IL1B (PN 62IL1PEB)	5.22	1.31%	54.65	0.67%
IL1B Control	Cisbio human IL1B (PN 62IL1PEB)	5.36	0.98%	55.09	0.95%
1X LANCE Detection Buffer	PE LANCE Ultra KinaSelect TK (PN TRF0301-D)	5.18	0.96%	50.94	1.07%
ULight-TK Peptide	PE LANCE Ultra KinaSelect TK (PN TRF0301-D)	5.32	1.98%	52.03	0.61%
Eu-anti-phosphotyrosine (PT66)	PE LANCE Ultra KinaSelect TK (PN TRF0301-D)	5.21	1.19%	53.24	0.57%
Ave of all reagents:		5.44	1.31%	54.22	0.95%

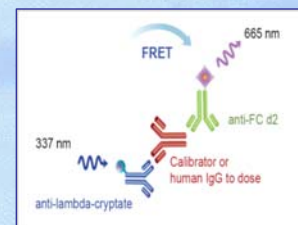


Stroboscopic image of acoustic droplet ejection (2.5 nl droplet). Typical precision of <3% CV and accuracy of <9% error on the Echo system ensures reliable data quality in dispensing of reagents from Cisbio HTRF kits.

3. Antibody screening with HTRF

Assay Principle

The Cisbio screening assay detects human IgG of the kappa or lambda isotypes in a sandwich immunoassay using homogeneous time-resolved fluorescence (HTRF) detection technology. Human IgGs are recognized by the anti-human kappa or lambda isotype labeled with europium cryptate (donor) and the anti-human Fc labeled with XL665 (acceptor). Upon recognition and excitation, the europium cryptate transfers energy to the XL665 acceptor which emits fluorescence.



Assay setup

Human Ab was titrated from 25 ng/well to 0.1 ng/well in two fold dilutions

+110 nl Eu Cryptate with ADE

+100 nl of XL665 acceptor with ADE

+ 40 µl of 50 mM PBS, 0.1% BSA, 0.1% KF buffer with Pipetman

Incubate at room temperature for 1-2 hours, read on RUBYstar HTRF reader (BMG)

Results

Table 2 shows a titration of human Ab, the average ratio of 665 nm/620 nm, standard deviation, assay result CV, number of wells, the Delta Force percentage and the Z factor. The results of this human IgG primary screening assay demonstrate that using the Echo liquid handler to prepare biochemical assays maintains high quality data, and offers further miniaturization to reduce reagent cost with the ability to dispense in nanoliter volumes.

Ab (ng/well)	Ratio 665nm/620nm AVE	SD	CV	n	Delta F%	Z Factor
25.00	24.12	1.01	4.2%	8	1944.27	0.83
12.50	22.73	0.52	2.3%	8	1826.43	
6.25	19.66	1.03	5.3%	8	1566.24	
3.13	16.79	1.76	10.5%	8	1323.62	
1.56	11.47	0.46	4.0%	8	872.53	
0.78	7.96	0.86	10.8%	8	575.12	
0.39	5.07	0.31	6.0%	8	329.92	
0.20	3.56	0.42	11.8%	8	201.78	
0.10	2.37	0.22	9.3%	8	100.91	
0	1.18	0.13	10.9%	8		

4. Conclusions

- The Echo liquid handler delivers a wide range of aqueous-based reagents with very low CV's and high accuracy.
- Multi-fluid flexibility enables total assay assembly on a single Echo instrument.
- Assays prepared using the Echo system maintain very high Z factor.
- Low-volume transfers enabled by ADE allow for further assay miniaturization and reagent savings.
- ADE offers a tipless solution to assay setup, saving costs and reducing waste.