Precise and Accurate Transfer of Kinase Enzymes Using Acoustic Droplet Ejection

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1. ABSTRACT

Aqueous enzyme solutions have historically been problematic to dispense in tip-based liquid handlers. Enzymes are often supplied in solution containing glycerol. Glycerol increases the viscosity of the solution, and decreases tip-based pipette performance as the sample tends to stick to both inside and outside of the tip. This reduces accuracy and increases imprecision in low-nanoliter volume transfers. Furthermore, aqueous enzyme solutions cause the meniscus within the well to lay highly tilted in some wells especially after centrifugation of well plates. The Echo® 555 liquid handler (Labcyte Inc.) utilizing acoustic droplet ejection (ADE) technology was used to accurately and precisely dispense highly viscous aqueous enzyme solutions. With the ability to recognize and adjust for tilted meniscus, the Echo 555 liquid handler was able to transfer 50 to 200 nanoliter volumes with assay CVs ranging from 3.2% to 4.6%. Use of ADE reduced valuable enzyme waste through elimination of tips while improving accuracy and precision. These experiments also open possibilities for total assay assembly using tipless, touchless high-throughput acoustic droplet ejection technology.

2. Material and Methods

Kinase enzymes typically stored in buffers containing: 20-50mM Tris, 100-150mM NaCl, 0.01-0.05% Tween 20, Triton 100, or Brij 35, 10-50% glycerol, 1-3mM DTT, and other chemicals such as beta-mercaptoethanol, EDTA, EGTA, or benzamidine were obtained . These were diluted with 100mM HEPES buffer. The enzyme was loaded into two wells of a 384-well polypropylene Echo qualified source plate (Labcyte Inc.) at 45 µL per well. Using the Labcyte Echo 555 liquid handler and Echo Cherry Pick or Echo Plate Reformat software (Labcyte Inc.), 50 nL, 100 nL, and 200 nL volumes were transferred from the two source wells to 6 rows, 5 rows, and 5 rows, respectively of each volume into a 384-well destination plate, either empty or pre-filled. The pre-filled plate was filled with 5 µL of substrate/ ATP, and the empty plate was filled with 5 µl of substrate using a 384-channel tip-based liquid handler after the ADE transfer. The plates were then mixed using a few different methods and/ or incubation times, and then sealed. Incubation was performed at 25° C (room temperature) for 90 minutes. A stop buffer of EDTA was added and phosphorylation of fluorescently-labeled peptide was analyzed using a Caliper Life Sciences LC3000 system.

Acoustic Droplet Ejection: Move Liquids with Sound™

Acoustic Droplet Ejection (ADE) uses focused ultrasonic energy to eject small droplets from a liquid (Fig. 1.) The technology can be used to eject droplets smaller than one picoliter and as large as 10 µL. Larger volumes can be transferred as multiple drops. ADE requires no tips, pins or nozzles, saving consumables and waste costs. With no contact between the ejection mechanism and the ejected sample there is no chance for cross-contamination. ADE delivers superior precision and accuracy for a wide range of biological applications, including siRNA screening, compound screening with biochemical and cell-based assays, PCR reactions and assay development.
See www.labcyte.com for additional information on ADE.

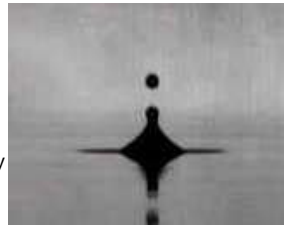

Figure 1. *Stroboscopic image of ADE*

The Echo Plate Reformat Software.

Echo Plate Reformat software simplifies a wide variety of plate transfer functions – plate replication, up-stack/down-stack plate reformatting, and custom regional mapping. An enhancement from the original Echo graphical user interface, this Echo application software offers great flexibility in specifying the number of source plates, transfer regions and transfer volumes. The drag-and-drop interface combined with color-coded plate preview simplifies the setup process for even the most complex cross-pooling protocols.

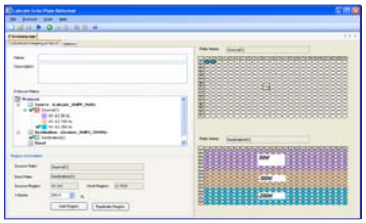

Figure 2. *Screenshot of Echo Plate Reformat software*

3. Phosphorylation of Peptide

Kinases transfer a phosphate group from ATP to specific substrates (fig 3). When the substrate is labeled with a fluorescent tag, the reaction can be tracked by modular systems such as the Caliper Life Sciences LC3000 equipped with separation and a detection unit. An electropherogram is generated as the substrate and its products are separated (fig. 4)

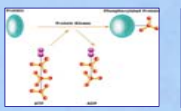

Figure 3.

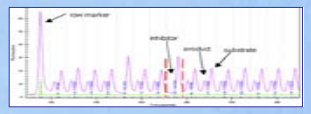

Figure 4.

Results

Multiple mixing protocols were tested to ensure optimum mixing of assay components. Table 1 shows the average assay plate CV for each dispense and mixing parameter. It was concluded that dispensing into an empty plate coupled with 30 seconds of shaking and centrifuging produced the lowest assay CV's. Table 2 shows the assay results for three different total assay volumes, including phosphorylated product ratio along with the standard deviation, and assay CV at each volume of dispensed kinase enzyme. These results 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.

Dispense and Mixing Parameters	Ave. Assay Plate CV%
Dispense into empty plate, 30 second shake	4.6
Dispense into empty plate, 30 second shake	4.2
Dispense into empty plate, 5 min. shake	9.0
Dispense into substrate solution mixed using Bravo and centrifuged	6.2
Dispense into substrate solution with 30 sec. shake and centrifuged	3.2
Dispense into substrate solution with 2 min. shake (no centrifuge)	42.9

Table 1.

	50nl	100nl	200nl
Average	0.498	0.566	0.517
Standard Deviation	0.020	0.027	0.022
% CV	4.106	4.728	4.296

Table 2.

4. Conclusions

- The Echo liquid handler transfers kinase enzyme with high precision.
- Proper mixing parameters coupled with centrifuging ensures reliable assay data.
- Low-volume, high-speed transfers enabled by ADE allow for further assay miniaturization and reagent savings while maintaining short assay setup times.
- ADE offers a tipless solution to assay setup, saving costs and reducing waste.
- ADE is compatible with a wide range of fluid types, enabling total assay assembly and miniaturization for many applications.

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