

Miniaturizing Singulex[®] Erenna[®] Assays using the Curiox Biosystems DropArray[™] System

As biomarker discovery studies advance, the field has been enhanced by the use of suspension bead assays and the single molecule detection capabilities of the Singulex[®] Erenna[®] assay system. However, this technology has some significant limitations when using precious clinical samples or working with animal models.

- Larger sample volumes –
Requirement of 100 µl/well
- Multiple plate transfers –
Burdensome workflow
- High cost of reagent –
\$800 - 1,500 for one 96-well plate

The Curiox DropArray[™] System is the perfect enhancement for the Singulex Erenna Assay System. Using the DropArray (DA) wall-less plate, standard Singulex reagents are added at 1/10th volume (i.e., for example 10 µl magnetic beads instead of 100 µl), and sample at 1/10th to 1/5th volume (for example 10 µl to 20 µl instead of 100 µl). Furthermore, a DropArray plate enables a unique convenient one-step bead transfer and precise transfer of eluted buffer during the assay, minimizing experimental error. Figure 1 (A-C) shows the unique DA-bead 96-well wall-less plate, the Curiox LT-MX washing station and a comparison of conventional microtiter plate vs. the DropArray workflow using the Erenna system.

Figure 1. (A) DA-Bead 96 well. (B) LT-MX washer (C) Conventional vs DA-Bead Singulex Workflow

- (A) DA-Bead 96 well plate holding 5µl drops/well respectively.**



- (B) Curiox LT-MX washer for DA-Bead plate**



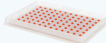

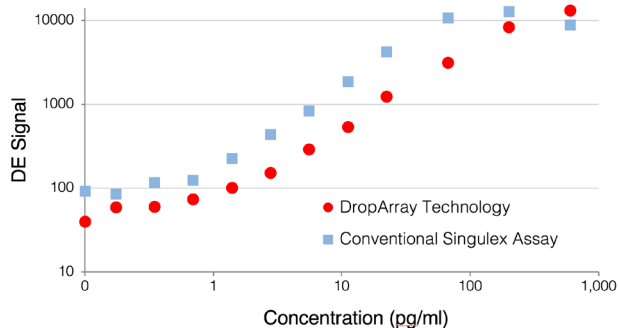
	Conventional Workflow	DA-Bead Singulex Workflow
1	Addition of Beads (100 µl) (1.7 x 10 ⁵)	Addition of Beads (10 µl) (1.7 x 10 ⁵) 
2	Addition of Sample/Standard (100 µl)	Addition of Sample/Standard (10 µl)
3	Orbital Shake/Incubate 1H	Vortex/Incubate 1H
4	Wash 1X	Wash 4X (LT-MX) 
5	Addition of Antibody (20 µl)	Addition of Antibody (5 µl)
6	Orbital Shake/Incubate 30 min	Vortex/Incubate 30 min
7	Wash 4X	Wash 5X (LT-MX)
8	Manual transfer to 96-well plate	Magnetic transfer to new DA-Bead Plate
9	NA	Wash 1X
10	Elute with 10 µl/well Buffer B	Elute with 11 µl/well Buffer B
11	Orbital Shake/Incubate 10 min	Vortex/Incubate 10 min
12	Transfer to 384-well plate	Transfer to 384-well plate
13	Read on Erenna [®] System	Read on Erenna [®] System

Figure 2. Equivalence Curve comparison: Microplate (100µl) vs DA-Bead cTn1 (10µl)



In addition to workflow and sample size improvements, adding DropArray to the Erenna system offers improvements in sensitivity. Figure 2 shows a comparison of a Singulex assay (cTn1) on a traditional microtiter plate vs. the same assay on a DropArray-bead plate. The DropArray plate showed improvements in sensitivity using 1/5th of the sample volume.

The DropArray system is elegantly designed to miniaturize magnetic bead-based assays such as Singulex and Luminex® platforms. Using Singulex cardiac troponin assay (cTn1) assay, the DropArray miniaturized workflow resulted in precision and accuracy analysis in line with conventional methods with intra assay CV% below 20% and recovery within acceptable range of 70-130% (Figure 3 & Table 1). DA-Bead offers reliable sensitivity with an LLOQ of 0.17-0.35 pg/ml, similar for both MicroPlate and DA-Bead Plate while maintaining excellent reproducibility.

DA-Bead offers excellent improved well-to-well and plate-to-plate reproducibility and equal to or increased sensitivity with better dynamic range when using 20 µl of sample volume per well instead of 100 µl of sample.

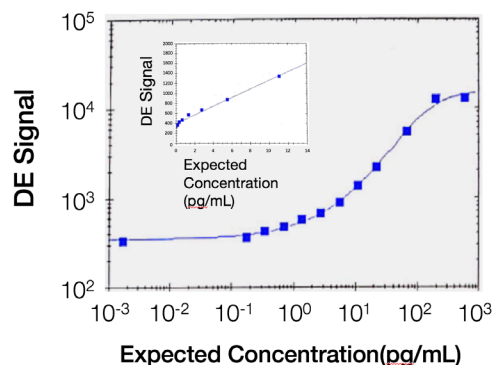
The DropArray System combined with the Singulex Erenna System is the next generation in Singulex assays. Together, they provide:

- Drastically smaller sample volumes, as low as 1/10th of that needed with Singulex alone
- Substantial decrease of reagent costs by 10-fold volume reduction.
- Increased sensitivity
- More robust assay to assay reproducibility
- Less complicated workflow

Table 1. Singulex cTn1 key performance parameters on DA-Bead

Expected Concentration (pg/ml)	Observed Concentration (pg/ml)	% Recovery	Intra-assay %CV
600	541.07	90	0.9
200	236.95	118	2
66.67	64.18	96	4.6
22.22	21.43	96	8.2
11.11	11.07	100	5.3
5.56	5.26	95	4.1
2.78	2.86	103	13.2
1.39	1.79	129	18
0.69	0.82	118	12.3
0.35	0.44	127	17.4
0.17(LLOQ)	0.12	70	5.3

Figure 3. DA-Bead Curve with Cardiac Troponin Assay(cTn1) minaturized with 20µl standards



$$\log(y) = \frac{1.65}{\left(1 + \left(\frac{5.04}{\log(x) + 3}\right)^{16.09}\right)^{0.29}} \quad R^2=0.9758$$