MINIATURIZATION OF SINGULEX®/ERENNA® BASED CYTOKINE ASSAY WITH WALL LESS 96 WELL PLATES

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PURPOSE:

Expansion in Biomarker discovery over recent years has been aided greatly by the use of suspension bead assay and single molecule detection capabilities of Singulex assay. Minimal volume requirement with such methodologies requires 50-100µl sample/well and can be a major bottleneck with precious limited clinical samples. Furthermore multiple plate transfer required by a normal Singulex workflow present a certain level of inconvenience. Here, we combine DA-Bead plate and Singulex assay and uncover new Singulex assay performance with 80% smaller volume than classically used methods.

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

C

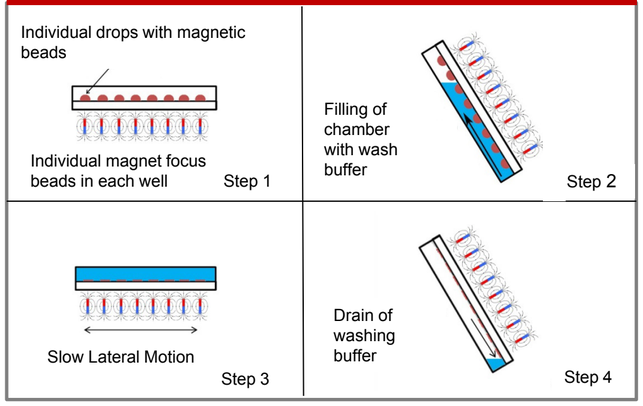
Step	Conventional	DA-Bead
1	Addition of Beads(100µl)(1.7x10⁶)	Addition of Beads(5µl))(1.7x10⁵)
2	Addition of Sample/Standard(100µl)	Addition of Sample/Standard(10µl)[1]
3	Orbital Shake/Incubate 1H	Vortex/Incubate 1H
4	Wash 1x	Wash 2X(LT-MX)
5	Addition of Antibody (20µl)	Addition of Antibody (5µl)
6	Orbital Shake/Incubate 30min	Vortex/Incubate 30min
7	Wash 4X	Wash 5X(LT-MX)
8	Manual Transfer to New 96 Well Plate	Magnet Transfer to New DA-Bead Plate
9	NA	Wash 1X
10	Elute with 10µl/well Buffer B	Elute with 11µl/well Buffer B
11	Orbital Shake/Incubate 10min	Vortex/Incubate 10 min
12	Transfer to 384 Well Plate	Transfer to 384 Well Plate
13	Read on Erenna System	Read on Erenna System

1: Presented data on figure 4 was generated by repeating step 2(2x 10µl)

METHOD:

DA-Bead is a wall less plate defined with 96 circular hydrophilic areas on an hydrophobic resin and follows classical microtiter plate grid format(Fig 1A). Each circular hydrophilic area receives conventional Singulex reagents such as magnetic beads/antibody/sample/standards in a minimal drop of 5-20µl volume for 96 DA-Bead plates. Following a highly similar workflow addition of reagents as in a conventional Singulex kit with conventional pipets(Fig 1C), DA-Bead is used sequentially on vortex shakers and washed in a fully automated station(Fig 1B and 2). DA-Bead to DA-bead plate transfer is performed conveniently and bypass a need for multiple pipetting steps required in a conventional method. Finally, beads on DA-Bead wall less plate are eluted out in a 384 well plate in a highly convenient manner before acquisition into Singulex reader.

Figure 2: Automated Washing sequence of DA-Bead.



RESULTS:

Using Singulex Erenna based kits for cardiac troponin-I, DA-Bead miniaturized workflow displayed precision and accuracy analysis in line with conventional methods with intra assay CV% below 20% and recovery within acceptable range of 70-130%(Figure 4 & table 1). Reliable sensitivity LLOQ reached 0.17pg/ml. DA-Bead offers excellent reproducibility well to well and plate to plate with 20µl of sample volume per well.

Figure 3: Equivalence Curve comparison: Microplate (100µl) vs DA-Bead cTn1 (10µl)

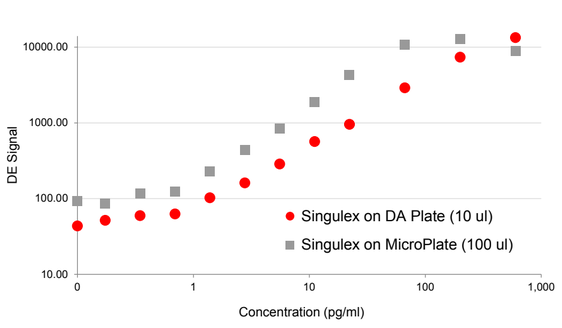
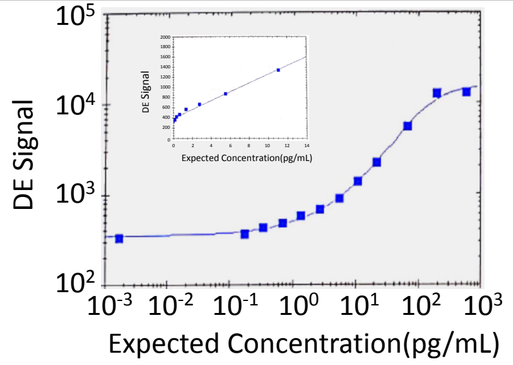


Table 1: Singulex cTn1 key performance parameters on DA-Bead

Expected Concentration (pg/ml)	Observed Concentration (pg/ml)	% Recovery	Intra-assay %CV
600.00	541.07	90	0.9
200.00	236.95	118	2.0
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
0.69	0.82	118	12.3
0.35	0.44	127	17.4
0.17(LLOQ)	0.12	70	5.3

Figure 4: DA-Bead Curve with Cardiac Troponin Assay(cTn1) minaturized with 20µl standards



$$\log(y) = \frac{1.65}{(1 + (\frac{5.04}{\log(x) + 3})^{16.09})^{0.29}}$$

R^2=0.9758

CONCLUSION:

With similar performance as conventional plates and an ease in workflow, DA-Bead is the next generation plate for Singulex assay which provides highest sensitivity performance with smallest sample volume.

- DA-Bead plates maximise Singulex assay performance with precious sample of 10 -20µl volume.
- DA-Bead follows similar Singulex workflow as conventional plates thereby requiring minimal learning.
- DA-Bead workflow generates significant Singulex reagents savings while maintaining optimal sensitivity.
- Curiox platform is optimally designed for small sample volume study with magnetic bead based assay such as Singulex and Luminex.