flexible liquid handling enables effective assay development for no-wash bead-based and cell-based immunoassays

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introduction

Traditional approaches to assay development often involve compromise due to limitations of automated liquid handling capability. Frequently resulting in many different and complex experiments, since it is not practical to investigate all assay variables in one plate. Consequently, the scope of assay development/optimisation may be significantly limited, for example, with respect to the number of different buffers and component concentrations tested. Liquid handling and experimental design tools often limit the plate density used in assay development to 96 well formats, which are not compatible for HTS. Here we present the dragonfly® discovery liquid handling system for assay development and HTS.

This flexible instrument, has been designed specifically for seamless transition between assay development and HTS. In assay development mode, it enables the creation of complex matrix layouts directly in 384 /1,536 well plates.

1. flexible assay development

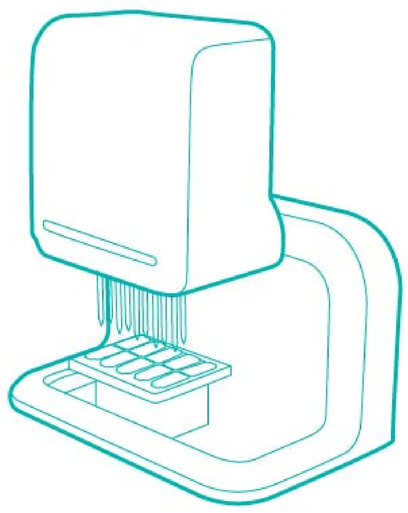

Fig 1. dragonfly discovery

key capabilities	
positive displacement tips	low dead volume no clogging or blocking of valves agnostic of liquid class accurate and reliable dispensing
non-contact dispensing	no cross-contamination high speed efficient use of tips
disposable tips	no cross-contamination minimal set up time low maintenance
high speed dispensing	rapid experimental plate set up (time savings)
independent channel control	easily create complex gradients and arrays
broad dynamic range (200 nL – 4 mL)	efficient use of precious reagents, high density plate compatibility
assay development design software (with DoE interface)	easily design and run complex experiments
automation friendly	develop, validate and screen on a common liquid handling platform
timed additions and plate lidded park zone	run time course assays prevent evaporation during incubation steps
manual or auto feed reservoirs	efficient use of reagents for assay development and HTS

2. bead based ELISAs

TTP Labtech's sol-R™ beads are fluorescently coded for use in multiplexed no-wash ELISA assays run on the TTP Labtech mirrorball fluorescence cytometer.

Fig 2. sol-R beads

sol-R beads:

- available as streptavidin-coated or carboxy coated toolbox kits
- up to 5 different codes for multiplexing

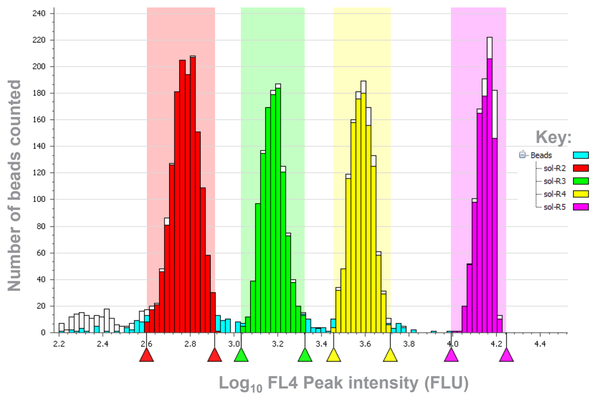

Fig 3. sol-R bead decoding

Fig 4. sol-R no-wash screening protocol

Simple workflow for no-wash assays involves adding 10 µl of a detection mixture (which contains beads and several detection components) to a well that already contains 10 µl of sample to be measured.

3. case study: no-wash cytokine assay optimisation

In order to obtain robust assay performance it is often necessary to determine optimal reagent conditions. In this example, the relative concentrations of biotinylated detection antibody and AlexaFluor® 488-conjugated streptavidin for use in the detection mixture must be determined.

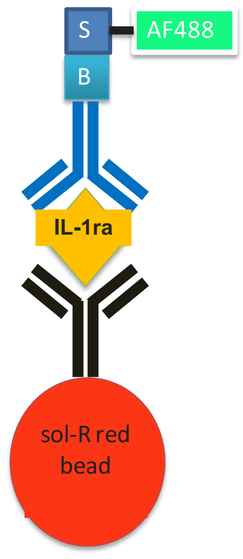

Fig 5. cytokine binding detection "fluorescence sandwich-ELISA" on sol-R bead

Converting from standard ELISA kits to fluorescence multiplexed assays require some level of assay optimisation. This has been achieved using dragonfly discovery.

4. assay optimisation matrix experiment

In this simple example, dragonfly discovery prepares a matrix plate comprised of a 2-fold dilution series of the cytokine and 20 different combinations of detection reagent compositions.

component	concentrations tested (units)
cytokine IL-1ra	500, 250, 125, 62.5, 31.25, 15.6, 7.8, 0 (pg/ml)
biotinylated detection antibody	25, 50, 100, 200, 400 (ng/ml)
Alexa-fluor 488 conjugated streptavidin	50, 100, 200, 400 (ng/ml)

Fig 6. range of analyte and detection reagent combinations dispensed by dragonfly discovery into the assay wells.

5. results

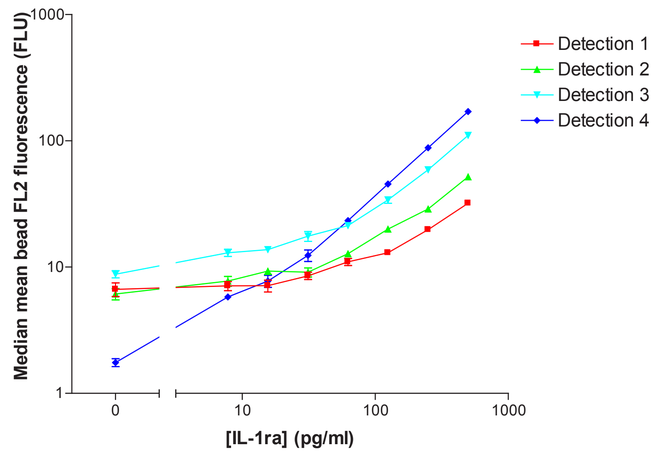

Fig 7. effect of detection reagent composition on cytokine standard curve

The intuitive dragonfly discovery software allows the user to create multiple standard dilutions and detection reagent conditions within the same experiment. This allows the user to readily select appropriate detection reagent conditions to give the desired assay window and/or assay sensitivity. In the example data shown above, clearly the combination of detection conditions in "Detection 4" give the best assay window and performance.

Once this optimisation step has been determined, the dragonfly discovery can be used in a screening environment to rapidly dispense detection mixture to the assay plates.

conclusions

TTP Labtech's dragonfly discovery provides a flexible approach to running complex matrix assay development protocols, which can easily transfer to high throughput screening laboratories using the same dispense technology and plate densities.

- simple workflows enable automated generation of multiple different assay conditions for more comprehensive and faster assay development and facilitate a more informed choice of assay conditions
- compatibility with all plate densities ensures seamless transitions from assay development through to high throughput screening
- disposable positive displacement tips and reservoirs ensure a wide range of compatible liquid viscosities, no risk of clogging or blocking or reagent cross-contamination