Appendix

Converting R&D systems ELISA DuoSet® to DELFIA technology

Step 1. Preparing your microplates

Indirect coating of Capture Antibody:

- Add 100 µL of antibody to each well
  - Reconstitute and store antibody according to data sheet
  - Determine the amount of ng/well from the R&D system protocol or your optimized values
- Incubate 2 hours plate shaker to ensure plates bind the capture antibody
- Wash x three times with 1x DELFIA wash solution
  - We recommend using a plate washer for consistency. If being done by hand it is simplest to dispense 200 – 300 µL of wash solution per well.

Direct adsorption of Capture Antibody:

- Add 100 µL of antibody to each well in DPBS without the presence of a carrier protein.
  - Reconstitute and store antibody according to R&D Systems data sheet.
  - Determine the amount of ng/well from the R&D system protocol or your optimized values
- Incubate overnight at room temperature
- Wash x 3 times with 1x DELFIA wash solution
  - We recommend using a plate washer for consistency. If being done by hand, it is simplest to dispense 200 – 300 µL of wash solution per well.
- Block the plate with 200 µL of DPBS + 1% BSA in each well
  - We recommend using Perkin Elmer CR84-100
- Incubate with blocking solution for at least 1 hour at room temperature on a plate shaker
- Wash x 3 times with 1x DELFIA wash solution

Step 2. Performing the assay

- Add 100 µL of standard analyte or sample to each well.
  - Prepare standards and any sample dilutions in DELFIA assay buffer
  - Reconstitute and store standard analyte according to data sheet
- Incubate 2 hours at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash x 3 times with 1x DELFIA wash solution
- Add 100 µL of biotinylated detection antibody to each well
  - Prepare working detection antibody solution in DELFIA assay buffer
  - Determine the amount of ng/well from the R&D system protocol or your optimized values
  - Reconstitute and store detection antibody according to data sheet
- Incubate 1 hour at room temperature on a plate shaker set to a slow speed (300 rpm)
- Wash x 3 times with 1x DELFIA wash solution
- Add 100 µL of Europium-Streptavidin (100 ng/mL)
  - Eu-SA solution stock concentration is 100 µg/mL
  - Prepare in DELFIA assay buffer
- Incubate 20 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
  - Cover the plate with a plate lid.
  - Do not cover the plate with TopSeal from this point forward
- Wash x 6 times with 1x DELFIA wash solution
  - The extra wash steps are necessary for removing any unbound Eu-SA
- Add 200 µL of Enhancement Solution and cover with a plate lid
  - If the plate is to be stored prior to reading, it is recommended to cover the plate and add Enhancement Solution just prior needing to read the assay.
- Incubate at least 5 minutes at room temperature on a plate shaker set to a slow speed (300 rpm)
- Read plate using TRF settings (see Table 2 in Materials and Methods). We recommend the EnVision system.
  - The developed signal will be stable for at least 24 hours when stored properly by covering tightly with parafilm. Note that seals or tapes with adhesives should be avoided after Enhancement Solution has been added to the plates.

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