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R&D SYSTEMS

Technical Note

Adapting DuoSet ELISA Kits to 384-well Plate Format

R&D Systems a Bio-Techne® Brand's award winning DuoSet™ ELISAs are widely used for the detection and quantification of various analytes in biological samples. These ELISAs offer robust and sensitive measurements, allowing researchers to study protein expression, biomarker profiling, and signaling pathway analysis. To enhance the efficiency of sample analysis, improve sample comparison accuracy, and conserve valuable samples, the adaptation of DuoSet ELISA kits to 384-well plates for high-throughput testing is highly beneficial.

Increased Sample Analysis per Plate:

By transitioning from traditional 96-well plates to 384-well plates, the capacity for sample analysis significantly increases. With four times the number of wells available, researchers can simultaneously analyze more than 160 samples in duplicate per plate, compared to only 40 samples in duplicate when using a traditional 96-well assay. This capability substantially improves laboratory throughput, reducing the time and resources required for large-scale experiments. Increased sample analysis per plate also facilitates parallel processing of controls, replicates, and experimental samples, ensuring efficient utilization of laboratory resources.

More Accurate Sample Comparison:

Utilizing 384-well plates enables all samples to be run on the same plate, ensuring a direct and accurate comparison between samples. By avoiding inter-plate variability, which may arise due to variations in sample handling, plate coating, and other factors, researchers can obtain more reliable and precise data. This enhanced comparability is particularly crucial for studies involving multiple treatment groups, time-course experiments, or longitudinal sample analysis. The consistent plate-to-plate results eliminate potential confounding factors, enhancing the statistical significance of experimental observations.

FIGURE 1. Human IL-6 Standard Curve

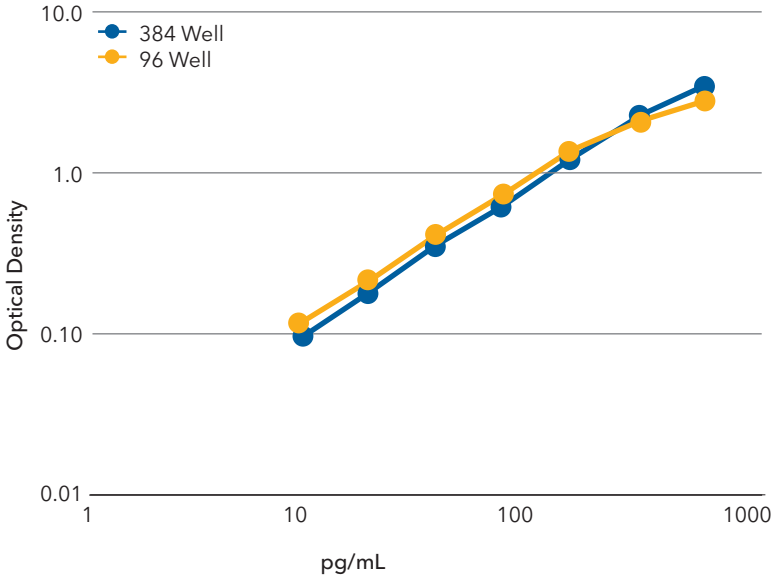
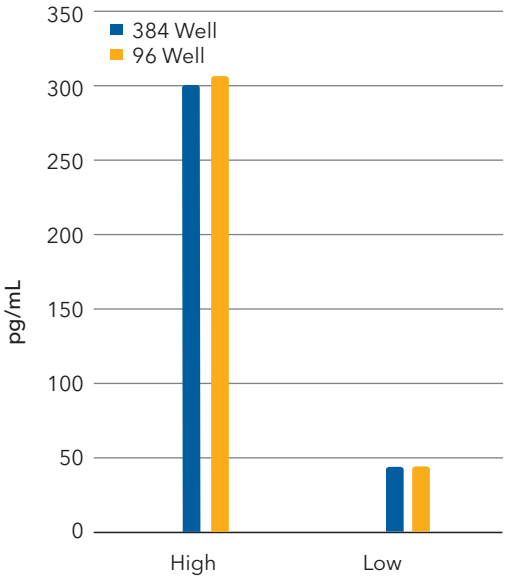


FIGURE 2. Human IL-6 Control Values



Standards (**FIGURE 1**) and Controls (**FIGURE 2**) were evaluated on 96 and 384 well plates using Human IL-6 DuoSet ELISA kit, catalog #DY206, with Ancillary Reagent Kit 2 for both plate types (DY008B and DY008B/384). Comparable results were obtained, affirming the adaptability of DuoSet ELISA kits across both plate formats.

Conserving Precious Samples:

High-throughput testing with DuoSet ELISAs in 384-well plates offers the advantage of reducing sample volume requirements. The smaller well size allows for substantial sample volume conservation while maintaining the necessary sensitivity and specificity of the assay. In a 96-well plate, 100uL per well is needed compared to just 25uL per well in a 384-well plate. This conservation of 75 uL per well is especially valuable when working with rare or limited sample types, such as clinical specimens or precious biological materials. Researchers can maximize the use of their samples, minimizing the need for additional collection or purification steps.

GENERAL ELISA PROTOCOL

The following is a suggested protocol to be used with our DuoSet ELISA kits in a 384-well format. Additional optimization may be required depending on your sample type.

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 384-well microplate with 25 µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (100 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 75 µL of Reagent Diluent to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Conclusion:

Adapting DuoSet ELISAs to 384-well plates provides numerous benefits for high-throughput testing. Researchers can increase sample analysis per plate, improving laboratory efficiency and reducing experimental time. The ability to run all samples on the same plate enhances sample comparison accuracy, eliminating inter-plate variability. Furthermore, the conservation of precious samples through reduced volume requirements is crucial for cost-effective and sustainable research practices. By leveraging the adaptability of DuoSet ELISAs to 384-well plates, researchers can streamline their workflow, enhance data reliability, and maximize the scientific impact of their studies.

Assay Procedure

1. Add 25 µL of sample or standards in Reagent Diluent, or an appropriate diluent, per well. Cover with an adhesive strip and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 25 µL of the Detection Antibody, diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 25 µL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 25 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 12.5 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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