CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

AlphaScreen®

Omnibeads™ Kit

Product number: 6760626D **Lot Number:** 3348339

Product Format: 6760626D: 1000 assay points

6760626M: 10 000 assay points

6760626R: 50 000 assay points

The number of assay points is based on an assay volume of 25 µL in 384-well assay plates using a final bead concentration of 20 µg/mL.

Manufacturing date: November 5, 2024 **Document version:** 1

Kit Components

Component	6760626D	6760626M	6760626R
Omnibeads Acceptor Beads at 5 mg/mL in 25mM Hepes, 100mM NaCl, 0.05% Kathon, pH 7.4	1 x 100 µL (6760132B)	1 x 1 mL (6760133)	1 x 5 mL (6760133B)

Product Information

Antibody/Protein: Omnibeads™ have been designed as a tool to identify instrument-related variability in Alpha assays. These beads contain all of the chemical components necessary for the generation of a strong Alpha signal without requiring the presence of AlphaScren Acceptor and Donor beads.

Stability: This kit is stable for at least 12 weeks from the date of manufacture when stored in its original packaging and the recommended storage conditions.

Storage: Store undiluted at 4°C protected from light. Freeze-thaw is not recommended and cause the beads to form aggregates.

Recommended Use: Omnibeads are light sensitive and should be handled under subdued or green filtered light conditions (< 100 Lux). All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters) can be applied to light fixtures. Vortex beads prior to use.

Quality Control

Maximum and Background signal are determined in an AlphaScreen® single concentration assay performed using a white 384-well Optiplate™ and read on an EnVision® detection instrument. We certify that these results meet our requirements

Maximum Signal:: 402716 counts

Recommended Assay Conditions (Instrument Validation)

For instrument verification testing, we recommend using Omnibeads at a concentration of 20 µg/mL. This concentration corresponds to the concentration of beads dispensed in a standard AlphaScreen assay. The volume of the Omnibeads per well depends on the plate format. We recommend using 25 µL/well in 384 plates, 100 µL/well in 96-well plates, and 7.5

µL/well in 1536-well plates. PBS or any physiological buffers (Tris or HEPES based) developed for AlphaScreen assays can be used.

Various plate set-ups can be used with the Omnibeads. Presented below is an example of an Omnibead test designed to verify a 96-well dispensing head used with a 384-well plate (Fig.1) – single and multiple addition steps. With this set-up, the plate average AlphaScreen signal for Q1 and Q4 wells should be equivalent. However, a lack of dispensing precision will be amplified in wells where multiple additions were performed.

Omnibeads Dilution:

1. Vortex thoroughly the Omnibeads stock suspension (5mg/mL)
2. Add 20 µL Omnibeads Acceptor beads to 4.98 mL 1x PBS, pH 7.2.
3. Mix well

Note: This procedure gives 5mL of a 20 µg/mL Omnibeads suspension. This volume is sufficient for the loading of half of the wells of the plate illustrated Fig.1.

Quadrant 1 (Q1): 25 µL of Omnibeads suspension per well

Quadrant 4 (Q4): 5 x 5 µL of Omnibeads suspension per well

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
B		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
C	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
D		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
E	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
F		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
G	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
H		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
I	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
J		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
K	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
L		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
M	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
N		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4
O	Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1		Q1	
P		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4		Q4

Figure 1. Schematic illustration of 384-well plate used for verifying a 96-well liquid handling dispensing head with Omnibeads.

Interpreting the Data:

The microplate is analyzed in a multiplate reader set for detection in AlphaScreen reading mode. Results from Q1 and Q4 are analyzed independently. Average counts, standard deviation (SD), and coefficient of variation (CV = [standard deviation / average counts]*100) are calculated for each quadrant. Typical results for Q1 wells are shown in Fig. 2.

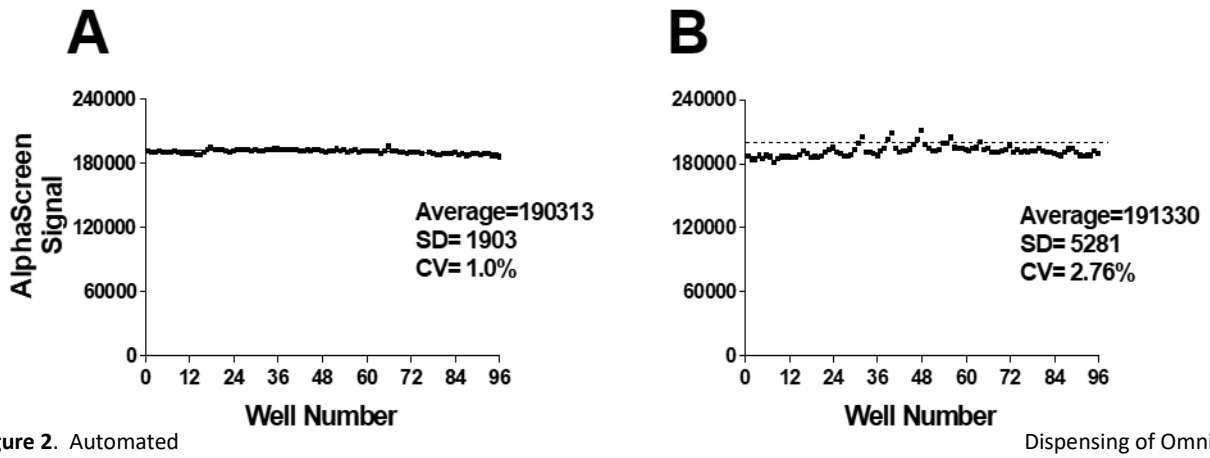

Figure 2. Automated Dispensing of Omnibeads in 384-well Optiplates. A volume of 25 µL of an Omnibeads suspension at 20 µg/mL was dispensed in all Q1 wells of the plates in a single addition. The plates were read with the EnVision™. A) Example showing the performance of a liquid handler dispensing the Omnibeads accurately. B) Example of a liquid handler with a few tips dispensing Omnibeads inaccurately. The dotted line represents the average signal plus two standard deviations. Wells above the dotted line are considered outliers.

Data analysis

The following situations indicate a malfunction of the liquid handler:

> Signal from individual wells of a plate differ from the average signal by more than two standard deviations. Counts higher than the average indicate leaks from the tips while counts lower than the average suggest a clogged tip or line (See Fig. 2B for a specific example).
>
> The CV of counts measured in Q4 wells is significantly higher than the CV of counts from Q1 wells. This is an indication of a sub-optimal dispensing precision. With a liquid handler deliver ing adequately, the CVs of the counts from both quadrants should be similar.
>
> The average signal measured from plates of a same series differs significantly. This indicates a general dispensing problem.

When these problems are detected, they can usually be corrected by following the troubleshooting procedures found in the instrument's instruction manual.

Evaluation of AlphaScreen Readers Accuracy

The most common problems encountered with AlphaScreen readers are faulty positioning of the plate inside the instrument, and a deficient temperature control. These situations will affect the assay results by increasing the signal variation from the plate.

1) Vertical (Z) positioning errors: Signal intensity varies as a function of the distance prevailing between the plate and the detector. Plates that are tilted by the uneven leveling of the instrument's plate positioner will show a drift in signal in a right-left or top-bottom fashion. This drift is caused by the slight differences in distance between the detector and the wells along the plate, and should be corrected.

2) Horizontal (X-Y) positioning errors: Accurate X-Y positioning is required for the proper alignment of the detector with the wells. A plate setup similar to the one shown in Fig.1 can be used for detecting incorrect X-Y positioning. Signal from Q1 wells is compared with the signal from adjacent empty wells. Counts in the empty wells should correspond to the plate background and be similar to the counts measured in an empty plate. If a significant level of counts appears in the empty wells adjacent to Q1 wells, the X-Y positioning of the multiplate reader requires adjustment.

3) Temperature Control: Temperature control issues occur when the instrument's reading chamber is not at the same temperature as the plate being read. All multiplate readers equipped with an AlphaScreen reading module contain a Peltier temperature controller, maintaining plates at a constant temperature during reading. The Peltier controller is meant to maintain

the temperature inside the instrument close to the ambient room temperature. If the temperature of the plate is very different from that prevailing in the room where the instrument is located (e.g. a plate that was just taken out from the cold room), the counts will increase during plate reading, as the plate's temperature equilibrates.

To determine if an AlphaScreen reader has a defective temperature controller, a plate with Q1 wells filled with Omnibeads is prepared. The counts of all Q1 wells are plotted in a scatter chart according to their reading order (Fig. 3). A slope of 0 indicates a constant temperature across the plate. A positive slope value indicates that the plate is warming up, while a negative slope value indicates that the plate is cooling down. If the slope cannot be corrected by pre-incubating the plate in the instrument for a few minutes prior to reading, an adjustment of the temperature controller may be required.

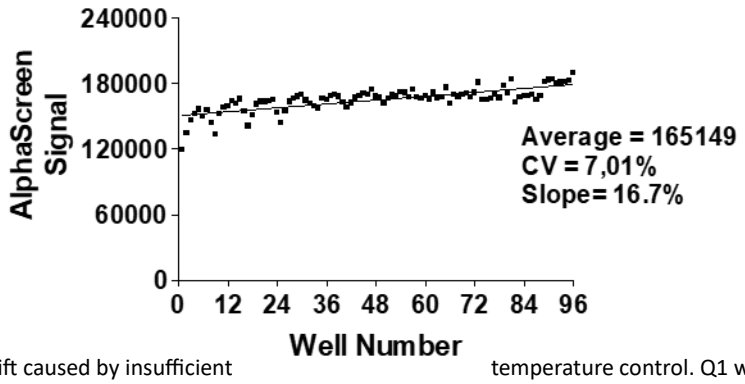

Figure 3. AlphaScreen signal drift caused by insufficient temperature control. Q1 wells of a 384-well OptiPlate were loaded with 25 µL of an Omnibead suspension at 20 µg/mL. The plate was deliberately incubated for 5 min at 4oC to recreate a temperature effect and read with an EnVision™. The positive slope value indicates that the plate was colder than the ambient temperature of the room where the reader was located.

Liquid Handler or Alpha Reader?

When an uneven signal pattern is observed in a plate loaded with Omnibeads, it is sometime difficult to determine if the problem arises from the liquid handler or multiplate reader. A simple way to discriminate between the two possibilities is to invert the plate in the reader, with well P-24 in the top-left position, and to read the plate a second time. If the count pattern is reversed (meaning that the same wells are giving abnormal counts), it is a dispensing problem. If the uneven pattern occurs at the same reading positions, then it is usually a reader problem.

Please visit our website for additional information on AlphaLISA technology at www.revvity.com

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