APPLICATION NOTE

Reliable and consistent AlphaLISA assay setup and execution using the AssayMate liquid handler.

Overview

Transitioning from Manual to automated AlphaLISA preparation

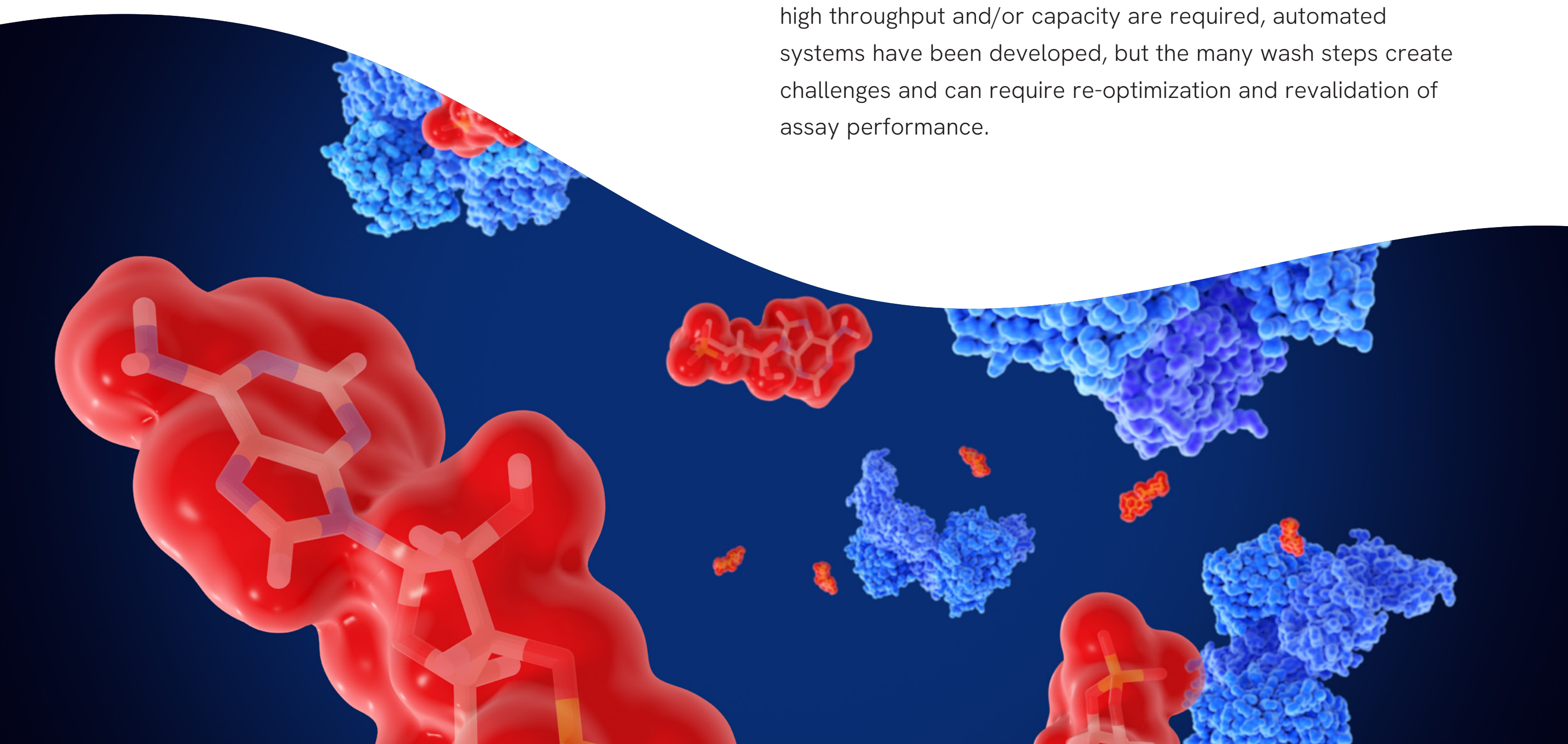
Traditional manual processing of reagents and samples presents some challenges that can limit laboratory productivity: high level of hands-on preparation time and maintaining high intra-assay precision during manual execution.

The AssayMate™ system is a budget-friendly, compact and user-friendly benchtop automated liquid handler, well suited, as a standalone system, for a range of sample and plate preparation tasks, freeing up valuable time for lab technicians and researchers.

Here, we demonstrate how the intraassay precision of the AssayMate system compares to manual processing of AlphaLISA™ reagents and samples, showing how the system can be used to reliably automate the range of AlphaLISA assay kits available from Revvity, saving hands-on time without compromising performance or reliability over manual process.

Introduction

Immunoassays are a mainstay for the quantification of a variety of bio-molecular analytes in drug discovery, drug development, and life sciences research laboratories. While ELISAs have traditionally been the most popular form of immunoassay, they are limited by the need to perform multiple wash steps. Where high throughput and/or capacity are required, automated systems have been developed, but the many wash steps create challenges and can require re-optimization and revalidation of assay performance.



To overcome these ELISA limitations, Revvity has introduced AlphaLISA, a novel, homogeneous immunoassay technology that eliminates wash steps. Compared to ELISA assays, AlphaLISA assays generally have a wider dynamic range and at least comparable sensitivity. AlphaLISA assays also can be scaled up from 96-well to 384-well format with no need for re-optimization. AlphaLISA panels are available in the following research areas: biologics, angiogenesis, cancer, cardiovascular, inflammation, metabolism, and neurodegeneration.

Using Revvity's family of automated workstations and microplate readers, AlphaLISA assays can be easily and reliably prepared, incubated, and analyzed without the need for complex and error-prone wash steps, time-consuming manual processing, or costly custom automation systems.

Preparation of AlphaLISA assays using the AssayMate benchtop automated liquid handler can be a cost-effective option for labs looking to transition from manual to automated workflows, or that have throughput needs which are better met by a more compact system. Moreover, the AssayMate system can be easily set up to process different assay types in a multi-user, multi-assay environment, thus providing a flexible solution for many laboratories.

Figure 1: AssayMate automated workstation

To demonstrate the performance of the Assaymate workstation in automating AlphaLISA assays, three AlphaLISA *SureFire*® assays (p-ERK1/2, p-Rb, p-STAT6) were prepared according to kit instructions, using a serial dilution series of positive control lysate as samples, on the AssayMate workstation. The experiment was repeated using the same kits and reagents as a manual, benchtop, process.

Assay results were compared after completion for readout values and precision (%CV) to show assay reproducibility and robustness.

Materials and methods

Reagents

AlphaLISA Sure Fire kits (p-ERK1/2, p-Rb, and p-STAT6) available from Revvity, were run as described in their kit inserts under subdued lighting conditions. Method of action for the assay kits is outlined in Figure 2. Assay workflow is outlined in Figure 3.

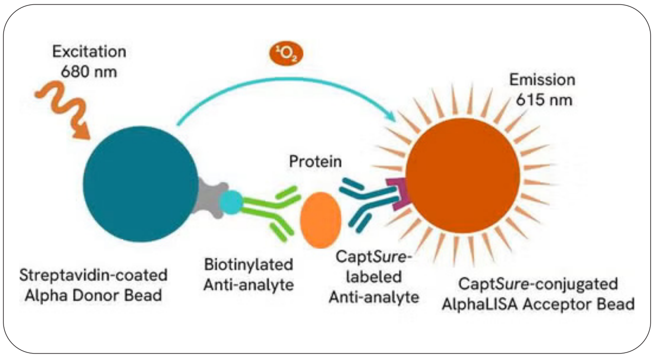


Figure 2: AlphaLISA Sure Fire reagent method of action

Instrumentation

Sample (control lysate) preparation for the automated portion of the test was performed with the AssayMate workstation, using control lysates provided in the assay kits with serial dilutions made with 1x lysis buffer in individual wells of a Revvity StorPlate 96-well v-bottom microplate. These samples, once prepared, were transferred to a Revvity ProxiPlate™ 384-shallow well Plus microplate, with each lysate dilution transferred to ten wells. Acceptor and Donor beads were added to this microplate by the AssayMate instrument when appropriate. Signal readouts were performed on a Revvity VICTOR Nivo™ plate reader.

This process was then repeated manually using the same reagent kits and microplate types.

Workflow

AlphaLISA workflow

- Create standards by serial dilution
- Distribute 10 µL of standards to microplate wells (10x)
- Add 5 µL acceptor bead to each assay well
- Seal microplate
- Incubate 60 minutes
- Unseal microplate
- Add 5 µL donor beads to each assay well
- Seal microplate
- Incubate overnight
- Unseal microplate
- **Read signal on VICTOR Nivo plate reader**

Figure 3: AlphaLISA workflow

Results

After an overnight incubation, microplates were read on the VICTOR Nivo plate reader with data collected and processed to determine mean signal for each concentration of lysate tested for each kit, standard deviation and standard error for each concentration, and finally, %CV calculations were made for each concentration for both the AssayMate workstation prepared and manually prepared plates. Results from these tests can be seen in Table 1.

Table 1: Summarized plate readout data. For each concentration tested sample size N = 10. As all three kits were run on a single microplate for those processed on the AssayMate system and manually, buffer-only blanks are shared for all three kits for each sample preparation method.

% Lysate in buffer	100	33.33	11.11	3.70	1.23	0.41	0.14	0.05	0
pERK1/2 AssayMate									
Average counts	20446.3	3583.3	634.9	208.1	137.2	114.5	104.8	145.8	66.4
Std deviation	244.5	107.3	29.6	30.3	20.9	13.5	17.9	77.6	12.2
Standard error	86.5	37.9	10.5	10.7	7.4	4.8	6.3	27.4	4.3
%CV	1.2	3.0	4.7	14.5	15.2	11.8	17.1	53.2	18.4
p-Rb AssayMate									
Average counts	39554.0	7679.1	1319.1	317.6	158.2	114.6	101.9	152.8	66.4
Std deviation	690.6	162.6	38.5	30.3	12.5	16.1	11.1	39.3	12.2
Standard error	244.2	57.5	13.6	10.7	4.4	5.7	3.9	13.9	4.3
%CV	1.7	2.1	2.9	9.5	7.9	14.1	10.9	25.7	18.4
p-STAT6 AssayMate									
Average counts	13043.6	2184.2	434.9	185.0	125.2	120.8	118.2	144.7	66.4
Std deviation	383.6	76.2	46.4	24.8	12.5	13.3	11.0	13.3	12.2
Standard error	135.6	26.9	16.4	8.8	4.4	4.7	3.9	4.7	4.3
%CV	2.9	3.5	10.7	13.4	10.0	11.0	9.3	9.2	18.4
% Lysate in buffer	**100**	**33.33**	**11.11**	**3.70**	**1.23**	**0.41**	**0.14**	**0.05**	**0**
p-ERK1/2 manual									
Average counts	20585.8	3532.8	648.3	226.7	158.9	126.4	117.1	173.6	91.2
Std deviation	833.2	310.4	35.5	58.9	34.4	40.2	46.2	99.9	10.1
Standard error	294.6	109.8	12.5	20.8	12.2	14.2	16.3	35.3	3.6
%CV	4.0	8.8	5.5	26.0	21.7	31.8	39.4	57.5	11.0
p-Rb manual									
Average counts	39426.3	7319.0	1325.1	337.9	166.9	129.0	124.1	152.8	91.2
Std deviation	1207.4	589.3	102.9	45.4	32.1	41.1	40.8	39.3	10.1
Standard error	426.9	208.3	36.4	16.1	11.4	14.5	14.4	13.9	3.6
%CV	3.1	8.1	7.8	13.4	19.2	31.9	32.8	25.7	11.0
p-STAT6 manual									
Average counts	13172.9	2436.2	454.8	175.5	139.3	125.4	130.9	167.5	91.2
Std deviation	400.1	453.2	47.6	31.6	24.9	43.7	32.7	47.4	10.1
Standard error	141.4	160.2	16.8	11.2	8.8	15.4	11.6	16.8	3.6
%CV	3.0	18.6	10.5	18.0	17.9	34.8	25.0	28.3	11.0

Summary

Our testing has shown AlphaLISA assay plates processed on the AssayMate automated liquid handling workstation provided consistently more precise results than manual process with the same kit reagents, with no appreciable difference in overall signal readouts.

This highlights the AssayMate workstation's utility as a viable tool in the overall AlphaLISA screening workflow, providing reliable, repeatable assay results and freeing time for lab technicians to engage in other tasks.

Discover more application notes.

For research use only. Not for use in diagnostic procedures.

2761500