Development of a cost effective and robust AlphaScreen® platform for HTS application

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The use of AlphaScreen® detection has allowed researchers to examine a wide variety of molecular interactions for use in high-throughput screening. However, the cost of Alpha reagents can often be prohibitory for extended screening campaigns or for young investigators with limited funding. To reduce assay costs, many labs have focused on miniaturization, while there have been limited efforts to scale down Alpha reagents. Thus, we describe the optimization of an AlphaScreen detection platform by systematically reducing the Alpha reagents down to 2.5 µg/ml beads, without compromising assay integrity. We demonstrate that reducing bead concentration reduces detection costs substantially while yielding robust statistics. We believe this simple new protocol will enhance the future utilization of AlphaScreen technology in high-throughput screening.

The use of the commercially available Amplified Luminescent Proximity Homogeneous Assay Screen (AlphaScreen®) detection platform has allowed researchers to examine a wide variety of molecular interactions including DNA, RNA, protein, peptides, and sugars [1]. The AlphaScreen detection platform is a mix-and-read assay platform that monitors the interaction between two analytes using bead-based proximity technology [2,3]. Although AlphaScreen has demonstrated superior

sensitivity and easy adaptation the cost of Alpha reagents can often be prohibitory for extended screening campaigns or for young investigators with limited funding. With a list price of more than US\$13,000 for 50,000 assay points, many labs have focused on reducing assay volume to generate a more cost-effective approach. Common protocols use 25–35 µl volumes with 20 µg/ml beads [4–6], following the manufacturer's recommendation. To reduce assay costs, labs have either reduced assay volumes in

384 well plates [7], miniaturized to 1536-well plates [4,8,9], or focused on reducing Alphabead concentration [7,9–11]. However, lowering the assay volume in 384-well plates or miniaturizing to 1536-well formats can reduce the quality of AlphaScreen assays and potentially affect hit detection [4]. By decreasing the assay volume, the error for each liquid dispense step can get compounded, which increases the assay variability. Thus, to examine more cost-effective methods without miniaturizing we initially sought to investigate how reducing bead concentration affects assay quality, as an alternative method for reducing assay detection costs [1,11].

Initial assays were performed in an assay buffer (assay buffer: 50 mM HEPES pH 7.4, 100 mM NaCl, 1.0 mg/ml BSA, and 0.05% CHAPS) using 10 nM ⁶His-biotin-peptide (positive control) or a biotin-labeled peptide lacking His residues (biotin-peptide; negative control). As shown in Figure 1A, assays were initially performed in white 384-well Optiplates (Perkin Elmer, MA, USA) with 20 µg/ml each streptavidin-coated donor and nickel chelate acceptor beads. The assay utilized a total volume of 20 µl, and reactions were allowed to incubate for 2 h before being read on an Envision plate reader equipped with a high-throughput AlphaScreen laser (Perkin Elmer). All reactions were performed in subdued light conditions (<100 lux) and at ambient temperature.

Initial examination of the bead concentration effect on assay quality showed that the signal-to-background ratio (S/B) dropped in a linear fashion when cutting the bead concentrations down from 20 µg/ml to 0.625 µg/ml, similar to what has been previously reported [11]. However, below 2.5 µg/ml beads, the S/B fell below 30, and was deemed unsuitable for further optimization (Figure 1B). Using 2.5 µg/ml beads would represent an eightfold decrease in the levels used for detection compared with the manufacturer's recommendation, and a fourfold decrease compared with commonly used cost-saving methods. Because reducing bead concentration

METHOD SUMMARY

We demonstrate that a reduction of bead substrate used for AlphaScreen® assay with minor re-optimization provides a robust and cost-effective alternative to traditional AlphaScreen methods without miniaturization or assay volume reduction. This new platform is suitable for high-throughput screening, and saves up to 90% on assay detection costs compared with the manufacturer's protocol.

Figure 1. AlphaScreen® assay optimization. (A) A diagrammatic representation of the Alpha assay principle used in this system. The positive control peptide contained both a His6 tag and a biotin tag, which interacts with the donor and acceptor beads, while a peptide that contains a biotin tag, but lacks the His6 tag serves as a negative control. (B) Dose-dependent decrease with Alphabeads (20 µg/ml to 0.625 µg/ml). The signal to background (S/B) ratio decreases linearly from 210 to 20. Note that at 2.5 µg/ml Alphabeads, the S/B is >50. (C) NaCl source effect on signal to background. (D) DMSO tolerance. DMSO was varied from 0 to 1%. The assay remained very tolerant to DMSO, even at reduced levels of Alphabeads. Percent control was calculated by normalizing alpha signal to that of 0% DMSO. Data point represents mean $\pm$ standard deviation. (E) The assay remains robust at lower Alphabead concentration. A total of 512 wells were dispensed to measure assay quality. 256 wells of positive control (⁶His-biotin-peptide) and negative control (biotin-peptide) were measured. The S/B, S/N, and z' were calculated to be 85.34, 14.92, and 0.79, respectively. This indicates that the assay is still robust enough for at a reduced Alphabead concentration of 2.5 µg/ml.

decreases overall Alpha signal and S/B is the primary criteria determining assay quality, it was critical to minimize the background to as low as possible. Individual assay components were first examined by varying catalog numbers or lots of HEPES, BSA and CHAPS, but no negative effects on assay quality were observed (data not shown). Interestingly, it was observed that a

broad range of S/B ratios (from 4.7 to 54.0) was generated when varying the source of sodium chloride. As shown in Figure 1C, the background signal (biotin-peptide) was changed by eightfold depending on the sources of NaCl, while the positive signal (⁶His-biotin-peptide) remained relatively unchanged. The increase in background signal reduces the S/B significantly, even

though the positive signal is relatively consistent. This dramatically degrades assay quality and demonstrated that the Alpha assay is sensitive to minor impurities in common buffer components, which is an important consideration while optimizing Alpha assays, particularly when reducing the overall signal.

Additionally, to ensure that assay stability had not degraded with lower Alphabead concentrations, we examined DMSO tolerance and room temperature stability. We found virtually no difference in DMSO tolerance as high as 1% and no variation in signal when incubating the reaction at room temperature for up to 2.5 h before adding Alphabeads (Figure 1D and data not shown). Collectively, this indicated that the overall reduction in Alpha bead concentration did not negatively affect assay stability.

To ensure that the assay is robust enough for high-throughput screening, we next examined how this assay performs in an format. We measured a total of 512 wells across multiple plates using positive control (⁶His-biotin-peptide) and negative control (biotin-peptide). The peptides were dispensed using a Janus Automated Workstation, while Alphabeads were dispensed using a MicroFlo Bulk Dispenser (BioTek, VT, USA). The MicroFlo dispenser is an automated reagent dispenser equipped with an eight tip cassette allowing speedy dispense of bulk reagents from a reservoir. Like other heterogeneous or high-salt reagents, Alphabeads often cause clogging of bulk dispenser tips when multiple plates were dispensed in an experiment, resulting in failure of uniform and accurate dispensing. While various cassette sizes (1, 5 and 10 µl) were available, the 5 µl cassette equipped with large bore (0.014 in) molded plastic tips was found to be most suitable. The 1 µl cassette had the lowest dead volume (5 ml), but the small bore tips (0.010 in) were prone to clogging. Among 5 and 10 µl cassettes equipped with large bore tips, the 5 µl cassette was chosen since it has a lower dead volume (7 ml) compared with the 10 µl cassette (10 ml), saving 3.5% in detection costs per day. The quality of the assay was measured by a statistical parameter – z' factor – which takes into account the mean and standard deviation values for the positive and negative controls [12]. As shown in Figure 1E, there was excellent separation between positive and negative controls, and the S/B, S/N, and z' were calculated to be 85.34, 14.92, and 0.79, respectively. This indicates that the assay is still robust enough for at a reduced Alphabead concentration of 2.5 µg/ml.

Table 1. A cost-effective alternative to miniaturization for AlphaScreen®.

Protocol	Assay points	Plate format	Bead dispense (μl)	Final bead conc. (μg/ml)	Detection cost (US\$)	Cost savings
Manufacturer	50,000	384-well	5	20	13,400	–
Traditional	50,000	384-well	10	10	5360	60%
New	50,000	384-well	10	2.5	1340	90%

and 0.79, respectively. Collectively, this demonstrates that the assay is robust and suitable for use in.

As shown in Table 1, this improved platform significantly reduced assay costs for a screen of 50,000 compounds to \$1,340, resulting in a savings of 90% compared with the manufacturer's recommendation, and 75% compared with commonly used cost-effective methods. This new method will provide an affordable platform for the continued use of AlphaScreen in campaigns, and for investigators with limited funds. Furthermore, given the abundance of Alpha reagents commercially available, this method can be applied to many other Alpha detection systems, since they all utilize bead-based proximity technology, as reported previously [1].

Financial & competing interests disclosure

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References

- PerkinElmer. A Practical Guide, AlphaScreen. Brochure 1–60 (2004).
- Ullman EF, Kirakossian H, Singh S Luminescent oxygen channeling immunoassay: measurement of particle binding kinetics by chemiluminescence. *Proc. Natl Acad. Sci. USA* 91, 5426–5430 (1994).
- Ullman EF1, Kirakossian H, Switchenko AC Luminescent oxygen channeling assay (LOCI): sensitive, broadly applicable homogeneous immunoassay method. *Clin. Chem.* 42, 1518–26 (1996).
- Von Leoprechting A, Kumpf R, Menze S Miniaturization and validation of a high-throughput serine kinase assay using the AlphaScreen platform. *J. Biomol. Screen.* 9, 719–725 (2004).
- Ungermannova D, Lee J, Zhang G High-throughput screening alphascreen assay for identification of small-molecule inhibitors of ubiquitin E3 ligase SCFSkp2-Cks1. *J. Biomol. Screen.* 18, 910–920 (2013).
- Wilson J, Rossi CP, Carboni S A homogeneous 384-well high-throughput binding assay for a TNF receptor using AlphaScreen technology. *J. Biomol. Screen.* 8, 522–32 (2003).
- Wigle TJ, Herold JM, Senisterra GA Screening for inhibitors of low-affinity epigenetic peptide-protein interactions: an AlphaScreen-based assay for antagonists of methyl-lysine binding proteins. *J. Biomol. Screen.* 15, 62–71 (2010).
- Ren Z, Tam D, Xu YZ Development of a novel β-secretase binding assay using the AlphaScreen platform. *J. Biomol. Screen.* 18, 695–704 (2013).
- Glickman JF, Wu X, Mercuri R A comparison of ALPHAScreen, TR-FRET, and TRF as assay methods for FXR nuclear receptors. *J. Biomol. Screen.* 7, 3–10 (2002).
- Yi F, Zhu P, Southall N An AlphaScreen-based high-throughput screen to identify inhibitors of Hsp90-cochaperone interaction. *J. Biomol. Screen.* 14, 273–281 (2009).
- Demeulemeester J, Tintori C, Botta M Development of an AlphaScreen-based HIV-1 integrase dimerization assay for discovery of novel allosteric inhibitors. *J. Biomol. Screen.* 17, 618–628 (2012).
- Zhang JH, Chung TD, Oldenburg KR. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* 4, 67–73 (1999).

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