A Low-Cost pNPP Assay as a High-Fidelity Alternative to Quanti-Blue for SEAP-Based NF-κB Activity Measurement

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Abstract

Background: Genetically engineered immune cell lines are widely used to study inflammatory signaling and immune modulation. While commercial assays such as InvivoGen's Quanti-Blue are commonly used, their high cost limits accessibility. To address this, we developed a low-cost SEAP assay using p-nitrophenyl phosphate (pNPP), reducing reagent costs from approximately $0.38/mL to $0.0165/mL (~23-fold reduction).

Objectives:. This study aimed to develop a cost effective replacement for commercially available SEAP immune assays and to evaluate the correlation and agreement between the standard Quanti-Blue assay and the pNPP-based assay to determine whether pNPP can serve as a cost-effective substitute for measuring NF-κB activity in stimulated THP-1 cells.

Method: Cells were exposed to nanoemulsified immunomodulatory drugs at three concentrations (2.5-25 μM) in the presence of 50 ng/mL lipopolysaccharide (LPS) to generate a range of NF-κB responses. Following 5–6 hours of incubation, supernatants were collected and incubated with either Quanti-Blue or pNPP substrates. Optical density was measured kinetically using a plate reader at the appropriate wavelengths.

Results: A significant correlation was observed between pNPP and Quanti-Blue assay results across multiple drugs and concentrations (Pearson r = 0.98, R^2 = 0.96, P < 0.001) over the full dynamic range (0–100% of maximal LPS response). Bland-Altman analysis demonstrated high agreement, with 95.8% of data points falling within the 95% limits of agreement.

Conclusions: These data demonstrate that the pNPP-based colorimetric assay provides a reliable, low-cost alternative to Quanti-Blue for quantifying SEAP activity and NF-κB signaling in engineered THP-1 monocytes, substantially reducing assay costs without compromising performance.

Introduction

Genetically engineered immune cell lines are widely used to study inflammatory signaling and immune modulation. The human monocyte line THP-1 has been modified to express secreted alkaline phosphatase (SEAP) under control of the NF-κB pathway, a central regulator of inflammation. SEAP activity is quantified through colorimetric assays based on phosphate cleavage from dye-linked substrates. While commercial assays such as InvivoGen's Quanti-Blue are commonly used, their high cost limits accessibility. This is particularly relevant with recent funding shortfalls in academic laboratories. To address this, we developed a low-cost SEAP assay using p-nitrophenyl phosphate (pNPP), reducing reagent costs from approximately $0.38/mL to $0.0165/mL (~23-fold reduction).

This study aimed to develop a cost-effective replacement for commercially available SEAP immune assays and to evaluate the correlation and agreement between the standard Quanti-Blue assay and the pNPP-based assay to determine whether pNPP can serve as a cost-effective substitute for measuring NF-κB activity in stimulated THP-1 cells.

Methods

SEAP-expressing THP-1 cells were cultured under standard conditions and viability was confirmed prior to experimentation. Cells were exposed to nanoemulsified immunomodulatory drugs at three concentrations (2.5-25μM) in the presence of 50 ng/mL lipopolysaccharide (LPS) to generate a range of NF-κB responses. Positive controls consisted of LPS alone, while negative controls included media-only and drug-containing emulsions without LPS to rule out nonspecific activation. Following 5–6 hours of incubation, supernatants were collected and incubated with either Quanti-Blue or pNPP substrates. Optical density was measured kinetically using a plate reader at the appropriate wavelengths. SEAP activity was normalized to maximal LPS-induced activation and expressed as a percentage of the LPS control.

Results

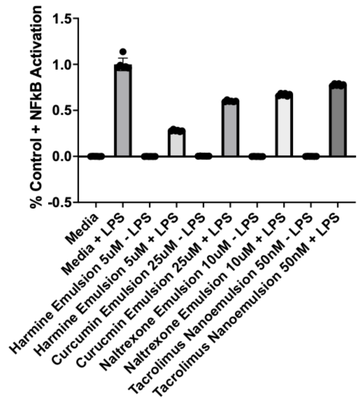

Figure 1: Representative readout from THP-1 NF-Kb SEAP assay using either Quanti-Blu or PNPP. For each sample, first the average measured optical density of the media blanks without LPS is subtracted from all samples measured (all wells from each group). Next, each corrected value is divided by the average value for the media + LPS group. This will make the media approximately 0% and the media + 100% and all other wells some % value of the media + LPS control. The graph shows mean values (bars) with all the individual wells (6) per group as dots. As expected, the emulsions with no LPS should have 0% or very close to 0% and the emulsions with drugs and LPS, some fractional value of the LPS control if they are working.

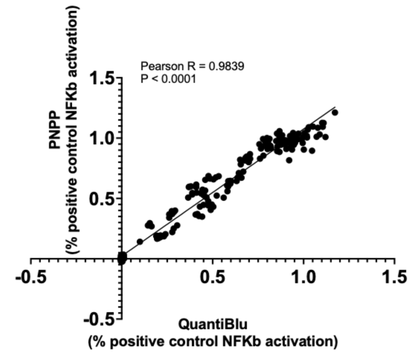

Figure 2: Correlation between all data points comparing % + media LPS control values measured by the Quanti-Blu assay (x-axis) vs. those measured using our modified PNPP assay (y-axis). The values were significantly correlated with a Pearson R value of 0.9839 and a P value < 0.0001

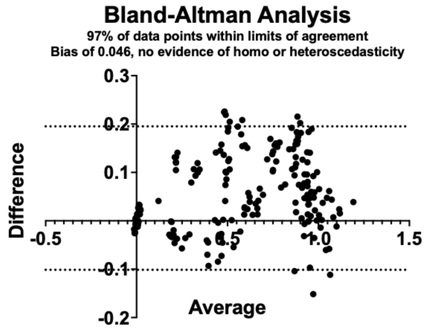

Figure 3: To demonstrate the interchangeability of the PNPP assay relative to the Quanti-Blu assay, Bland-Altman analysis was performed to demonstrate agreement between the values. 97% of the sampled data fell within the limits of agreement (-0.1016 to 0.193). There was a minimal systematic bias 4.6% PNPP relative to Quanti-Blu. These criteria made the PNPP assay suitable for our comparative studies and bench work analyzing nanoemulsion immune modulation and for replacing the more costly Quanti-Blu assay.

Discussion

Discussion: Our findings demonstrate that the pNPP-based colorimetric assay is a reliable, low-cost alternative to Quanti-Blue for quantifying SEAP activity and NF-κB signaling in engineered THP-1 monocytes. This assay substantially reduces costs without compromising performance. Strong correlations were observed between the pNPP and Quanti-Blue assays across multiple drugs and concentrations (Pearson r = 0.98, R^2 = 0.96, P < 0.001) over the full dynamic range (0–100% of maximal LPS response). Further verification by Bland-Altman analysis showed that 95.8% of data points falling within the 95% limits of agreement. These results support strong agreement between the two assays. The pNPP assay allows laboratories with limited resources to conduct SEAP-based immune assays while maintaining statistical performance.

Limitations: A limitation of this study was the use of a single cell line (THP-1), which may limit generalizability. The protocol used specific immune conditions that may not capture all assay actions. Bland-Altman analysis showed a small positive bias, with pNPP values averaging 4.5% higher than Quanti-Blue. Additional testing using other relevant inflammatory stimuli and at further time points of incubation would strengthen the statistical correlations and applicability of both assays.

Future Directions: Future studies should evaluate the pNPP assay in additional cell lines beyond THP-1. More experimentation with SEAP data-collection modalities and inflammatory pathways would broaden the assay's use in nanoemulsion studies. Longer-term experiments will help achieve greater assay consistency and cost-effectiveness.

References

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