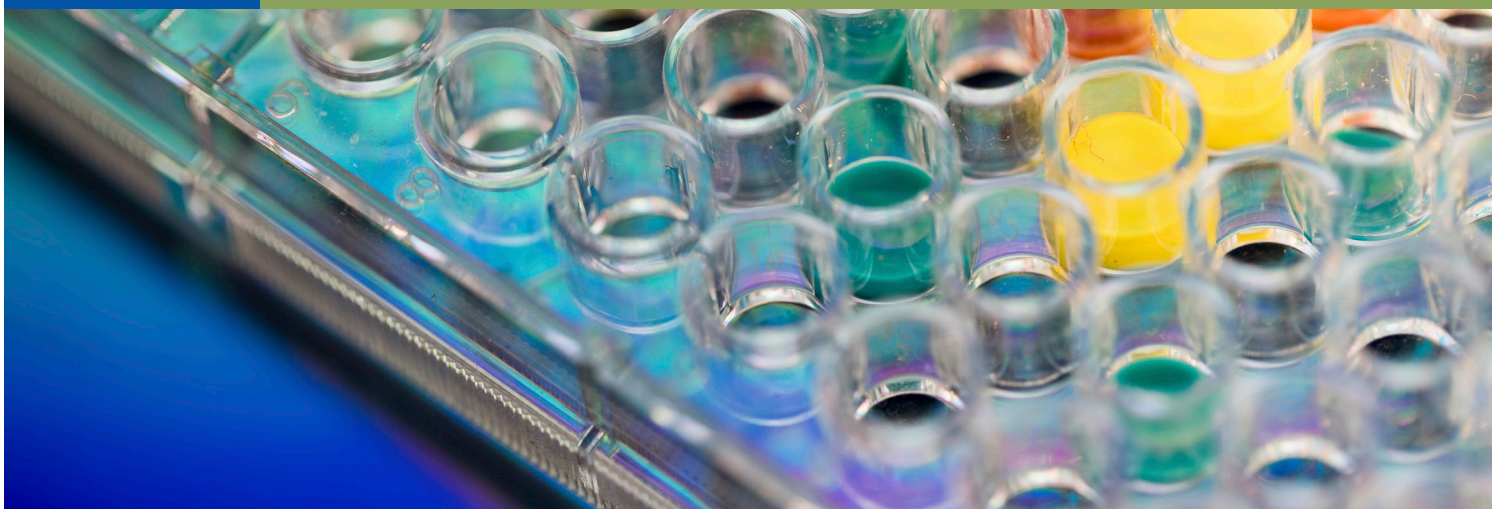
BRIEF

Seven Mistakes to Avoid in Assay Development



Key Considerations for Developing Biological Screening Assays

Introduction

In drug development and toxicity testing, the creation of test systems, or assays, in which you can evaluate the effects of chemical compounds on cellular, molecular or biochemical processes of interest, is a crucial first step to getting your drugs to market faster. However, developing these biological assays is not easy. There are a number of fundamental factors that influence outcomes, and an improperly designed assay can lead to the loss of precious time, samples and reagents. To help you develop assays that are specific, sensitive and robust, we've put together a quick guide in which we discuss some of the common challenges that may arise and how to avoid them.

Mistakes to Avoid

1. **Poor antibody selection:** Without the correct antibody, your immunoassay is in danger of failure. Selecting the right antibody and reagent is crucial – more details on that below.
2. **Using the wrong plate:** Often researchers choose a microplate from the shelf or pick solely based on cost without recognizing that the plate is an important component to achieve good results. The color, material and planarity of your microplate can make significant differences in assay performance and quality of data obtained, making it worthwhile to do a little research and find the best option.
3. **Over grown or poorly cultured cells:** This can lead to low viability or poor target expression, so it's important to ensure you have an appropriate amount of evenly grown cells in the plate.
4. **Not paying attention to the dynamic range of the assay:** If dynamic range isn't suitable, some data points could be below the limit-of-detection or above the saturation or hook point of the assay, leading to erroneous results. A large dynamic range allows the most data points to be within the lower and upper limit of detection of the assay without the need for diluting samples.
5. **Placing too much importance on the signal intensity or signal to background ratio of the assay:** What really matters is the information generated by the assay, for which the Z' value is a better indicator (i.e., a low signal intensity, low S/B, high Z' assay is better than a high signal intensity, high S/B but low Z' value). This is even worse when you are working in a regulated environment in which the absolute readings are required in QC documents and are difficult to amend.

PerkinElmer®
For the Better

6. **Not paying attention to your plate reader:** It is important to understand the actual capabilities of the instrument you have selected: What are maximum obtainable or reasonable signal levels? Can you trust a measurement point, or might it become unreliable over a certain range? Assuming the instrument itself is capable, you also need to be sure that the correct filters and/or optical settings are used. For example, excitation and emission filters each have specific bandwidths, and an incorrect combination can lead to increased background. Complimentary filter combinations must be carefully selected for optimal performance. Also, although many assay kits suggest reading "at" a given wavelength, the truth is a bit more complicated. The characteristics of the optical modules might differ slightly from what is written in the kit insert. So sometimes it is better to deviate from kit manufacturer's recommendation on the best assay wavelengths.
7. **Not performing systematic optimization of assay conditions.** Assay conditions such as buffer composition, pH, order of reagent addition and reaction time course need to be tested, with appropriate controls.

Critical Focus Areas for Assay Development

So there are a lot of things that can go wrong, but you can minimize that risk by focusing attention on some critical areas first.

Reagent quality: As you begin the development phase for immunoassays, you must first consider the selection and quality of your antibody. You should understand the availability of quality validated antibodies. While using only western validated antibodies is common, it also presents a roadblock. Reliance on antibodies that have only been validated for western blotting, or unqualified antibodies obtained from partnering labs, can lead to poor or non-selective binding.

For immunoassays made in-house, antibodies are typically difficult to use. Therefore, it's important to put much of your attention into the choice of the right antibody for your assay by checking for purity, concentration, specificity, and epitopes to avoid steric hindrance. The final developed assay should also be re-checked for specificity, sensitivity, and sample compatibility.

Similarly, high quality enzymes and recombinant proteins should be used for enzymatic and binding assays.

Predictability: It is vital that your assay be as predicative as possible of what the real situation will be in the patient. Assays must be performed with pharmacologically relevant concentrations of biological components to yield meaningful information. Technologies failing to achieve this are at risk of introducing biases that can lead to false positives/negatives.

Sensitivity, time and cost: Choosing a less sensitive and/or more time-consuming assay can lead to missed opportunities, ultimately impacting your overall productivity. In contrast, sensitive assays that are easy to perform help to improve cost-effectiveness.

Getting the Help You Need

Depending on the complexity of your assay and your current workload, outsourcing your assay or reagent development could potentially save you time and money in the long run – not to mention many headaches along the way. Custom assay services, such as PerkinElmer's OnPoint assay development service and "toolbox" and custom reagents, offer more flexible options, in addition to ready-to-use kits.

Whatever approach you choose, having a supplier that has the resources and expertise to partner with you on your assay development can help you avoid potential pitfalls, and get the quality results you need, faster.

About PerkinElmer

We offer state-of-the-art reagents, fully validated assay kits, biochemical and cellular assay technologies, plates, readers, automation and software, delivering a complete solution for your ever changing detection needs. By working with our experts, your scientists are able to devote less time to assay and reagent development and focus more on the research at hand, improving your lab's productivity. From assay development through whole animal imaging, we have you covered.

What's more, we also provide incredible support to our customers. Our Field Applications Specialists work directly with you in the planning stages, which allows us to highlight any potential pitfalls we might see further down the road.

www.perkinelmer.com/category/high-throughput-and-phenotypic-screening

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

014358_01 PKI