CRITICAL REAGENTS IN BIOASSAY
THEIR IMPORTANCE, CHARACTERIZATION AND STORAGE

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What are Critical reagents and why are they important

• Reagents that can curtail or alter the performance of assays
• Difficult to make, replace, acquire or substitute
• They can be any reagent identified by the user as critical to the life cycle of the assay.
• Critical reagents geared towards ligand Binding Assays
• Eg: Abs, special reagents like FBS, special buffers etc
• Generation of lots and materials that are also stable over the life cycle of the assay.
Critical reagents generation.

Points to consider

• L4 Global Harmonization Team
• US FDA and the EMA
• Critical reagents documentation
• In-house produced critical regent versus commercial source reagents
• Changing critical reagents
• Stability and storage of the critical reagents
Critical reagents documentation

• SOPs, documented analytical procedures, work instructions
• Reproducibility and consistency
• Types of critical information needed for documentation
• Characterization and qualification
• Handling and storage
• Side by side comparison or other appropriate documented procell to switch to another lot
• Pre-defined acceptance criteria for switch to another lot
In-house produced critical reagents versus commercially sourced reagents.

- Availability of reagents throughout the life cycle of the assay.
- Sufficient quantities
- Cost determines direction
<table>
<thead>
<tr>
<th>Availability and cost-effectiveness</th>
<th>Pros</th>
<th>Cons</th>
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<tbody>
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<td></td>
<td>• Custom in house reagents can be tailored to suit the assay design.</td>
<td>• Needs special planning and infrastructure for in-house reagent production</td>
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<td>• Independence from commercial sources</td>
<td>• In-house production of reagents (e.g., recombinant proteins/antibodies) requires significant investment in personnel (expertise) and capital</td>
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<td>• Cost-effectiveness and availability of in-house resources could be a major challenge</td>
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<td>Characterization</td>
<td>• Reagents can be extensively characterized for the intended use, and certificate of analysis can be customized.</td>
<td>• Reagent characterization can be time-consuming and expensive. Time is of essence in most developmental projects.</td>
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<td>• Lot-to-lot variability (especially in protein labeling) can be monitored and controlled.</td>
<td>• In-house expertise may be limited.</td>
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<td>• Stability data can be generated in-house.</td>
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<td>Ease of use</td>
<td>• Better control on life cycle of reagents.</td>
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<td>• Availability of a sufficient amount of the reagents can be exerted by better</td>
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## Commercially produced reagents summarized

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| **Availability and cost-effectiveness** | Readily available off-the-shelf reagents from a variety of vendors | • Availability of a particular reagent may be discontinued without prior notice  
• Changes to quality attributes without upfront notification might impact assay performance |
|                        | Custom reagent production is not limited to production of critical reagents (e.g., recombinant proteins, antibody, and antibody conjugates) in-house. These reagents can be produced by commercial companies according to customer needs | Lot-to-lot variability. Lot changes may meet vendor release specifications but may be a significant risk to consumer |
|                        | Specific reagents (e.g., recombinant proteins, antibody, and antibody conjugates) can be custom prepared | Lot change may reflect both conjugation (replace by “final product”?)) and starting materials |
|                        | Relatively inexpensive | Custom reagents are generally more expensive than off-the-shelf |
## Commercial produced reagents cont.\(^1\)

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<td><strong>Characterization</strong></td>
<td>Generally characterized for intended use, and certificate of analysis is provided</td>
<td>Certificate of analysis may not have all the characteristics that one would like to see. Potential lot to lot variability requires monitoring.</td>
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<td>Specific reagents can be characterized as per customer specification</td>
<td>Stability data are generally missing. It should be done in-house</td>
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<td><strong>Ease of use</strong></td>
<td>A wide variety of conjugated proteins can be obtained and tested for superior performance in the assay</td>
<td>May require considerable efforts to select a reliable reagent and a reliable vendor</td>
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<td>Calibrators available in preweighed quantities</td>
<td>Concentration assignments for preweighed calibrators vary widely varied, especially if the concentration is expressed in international units</td>
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<td>Premade buffers and assay diluents tested to work with critical reagents are readily available</td>
<td>Proprietary composition of specialty buffers (e.g., wash buffer) may create logistic issues</td>
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Changing critical reagents

• When and why.
• FDA guidelines for changing critical reagents
• Major or Minor changes.
• Requalification, optimization of assays
• Side by side studies
• 3 plate studies in parallel or over a period (you will have to explain that bullet point)
Stability and storage of critical reagents

- Conditions guaranteeing the stability of the reagents
- Various conditions provide varying degrees of stability
- Room temp. vs refrigerated
- -20 °C vs – 80 °C

Arrhenius equation; 
\[ k = A e^{-\frac{E_a}{RT}} \]

- K is the rate constant, A is the pre-exponential factor, Ea is the activation energy, R is the universal gas constant and T is the absolute temperature measured in Kelvin. (you will have to explain that bullet point)
Stability and storage of critical reagents

• Expiry date versus Retest date
• Scientific documentation over a period of time
• Systematic evaluation during the life cycle of the reagent
• Freeze thaw cycles, storage times and temperatures
• Something as simple as aliquotting
• Extensive controls and oversight on their storage.
• Useful tools: DoE, control charts
References


