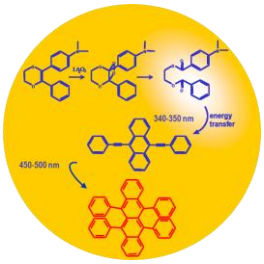
ALPHA TECHNOLOGY: QUESTIONS

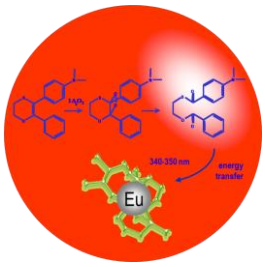
1- Which is the difference beween AlphaLISA and AlphaScreen acceptor beads? Which one would you choose to analyze serum samples, and why?

AlphaLISA beads contain Eu (emission at 615 nm, sharp peak), while AlphaScreen beads contain rubrene (emission at 570 nm, large peak). AlphaLISA are suggested for serum and plasma samples because their signal is not influenced by hemoglobin in case of hemolyzed samples.

rubrene



europium



2- For the conjugation of acceptor beads with an antibody, in which buffer should be the antibody and at which concentration?

Amine-free buffer, such as PBS or Hepes (Tris is not suitable protein conjugation on beads).

The required conc. of antibody is 1 mg/ml, to obtain a good conjugation yield.

3- What is the first step of the optimization of a customized immunoassay based on Alpha technology, and why is this step so important?

Generally, the first step of Alpha assay optimization (in case of customized assay) is antibody titration (for those antibodies that are not covalently conjugated to beads).

This step is critical to avoid Hook effect, i.e. to avoid saturation effects and thus to detect which antibody concentration provide the most intense Alpha signal. Slightly suboptimal antibody concentrations are normally chosen, to avoid the Hook side of the titration curve. Such optimization step is usually performed with a fix, intermediate conc. of analyte, in order to avoid saturation of the beads by the analyte. It is possible to use just one analyte concentration because of the vast dynamic range of analyte concentrations that can be detected by Alpha technology.

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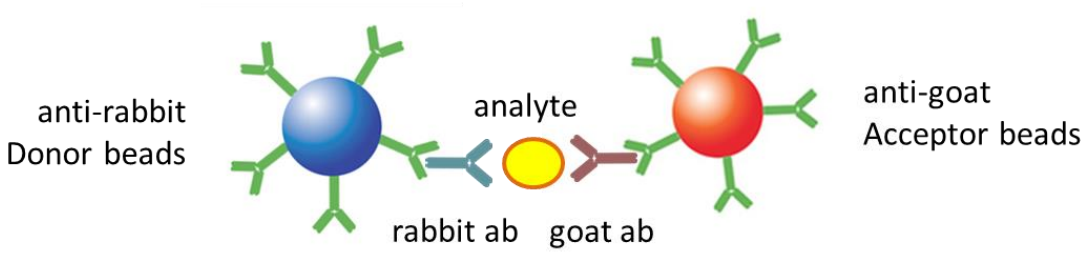
4- How would you quantify adiponectin by AlphaLISA, using these anti-adiponectin antibodies:
   - Antibody 1: goat polyclonal, 200 μg/ml in PBS, sodium azide 0.1%, gelatin 0.1%
   - Antibody 2: rabbit polyclonal, 1 mg/ml in HEPES, BSA 0.1%, glycerol 50%

   Draw your Alpha assay:

In this assay, it is not possible to use direct conjugation because both antibodies solutions contain carrier proteins (antibody biotinylation should be excluded for the same reason → you would biotinylated also carrier proteins!).
Antibody 1 is also too much diluted for conjugation procedures (also for biotinylation).

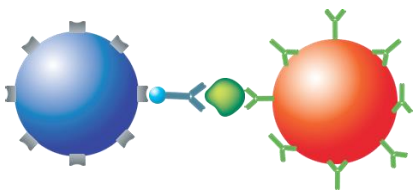
The best option here is to use secondary antibodies. Cross-titrations of their conc. will be required as first optimization step.
At the moment, Donor Beads are not available as anti-goat, thus we have to choose anti-goat Acceptor beads and anti-rabbit Donor beads.



5- How would you design an AlphaLISA immunoassay for leptin quantification with the following antibodies:
   - Antibody 1: goat polyclonal, 1 mg/ml in PBS, sodium azide 0.1%
   - Antibody 2: biotinylated rabbit polyclonal, 1 mg/ml in HEPES

   Draw your Alpha assay:

Antibody #2 is already biotinylated and we can use it with Streptavidin-coated Donor beads.
Differently from exercise #4, in this case it is possible to use direct conjugation for Antibody #1, because its solutions does not contains carrier proteins and has right concentration (1 mg/ml).
The best configuration here is the one below (advantage: little optimization required, only titration of Antibody #2).



6- How would you quantify cortisol using AlphaLISA and only one anti-cortisol antibody (rabbit polyclonal, 1 mg/ml in PBS) + biotinylated cortisol?
Draw your Alpha assay:

Since there is only 1 available antibody (cortisol is a small molecule), this is a competition assay.
We already have the "tracer-analyte" already suitable, too.
The antibody can be directly conjugated, as in exercise #5. The best configuration here is the one below (advantage: little optimization required, i.e. the titration of tracer-analyte to setup its ideal concentration for the competition assays).

7- Which parameters can be optimized when developing a customized AlphaLISA assay?

In addition to point #3, you can optimize incubation times, buffer composition (e.g. add 0.1-1% BSA to avoid unspecific binding and also to increase the sensitivity in some cases), order of reagent additions, beads concentration (rare cases), sample dilution (to avoid matrix effect), depending on the configuration of your assay.