UltraAmp™ reagents provide signal amplification in a variety of ELISAs

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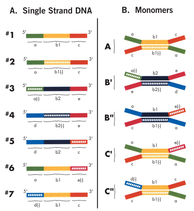
Abstract

Enzyme-linked immunosorbent assays (ELISAs) are widely used in disease diagnosis (Hepatitis, AIDS, Alzheimer's, etc), monitoring of environmental contaminants, and drug discovery (identification or monitoring of biomarkers). ELISAs are relatively cheap assays, and allow high throughput, but are often limited by sensitivity. To improve on the limits of detection of ELISAs, we have developed horseradish peroxidase (HRP) multi-labeled UltraAmp reagents, with up to 200 HRPs per molecule that can be targeted to the analyte of interest. UltraAmp reagents are DNA dendrimers conjugated with a variety of labels and targeting moieties. First, enzymes, haptens and antibodies are conjugated to oligonucleotides that are complementary to the outer layers of the dendrimer; then the DNA dendrimers are functionalized by hybridization with the modified oligonucleotides. We compared a variety of UltraAmp reagents (anti-biotin, anti-IL-6, anti-IL-1 alpha) against the standard streptavidin-HRP (SA-HRP) method for detection in interleukin-6 (IL-6) and IL-1 alpha (IL-1a) sandwich ELISAs as models for the development. The results showed that signal amplification was as high as 200-fold among UltraAmp reagents and antibody pairs compared to streptavidin-HRP (SA-HRP) detection. In most cases, UltraAmp reagents with greater number of HRP molecules attached gave greater signal with a slight increase in background. However, in some cases UltraAmp reagents with 50 HRPs performed better than reagents with 200 HRPs presumably due to their smaller size and hence better ability to reach the target. Overall, our results indicate that UltraAmp reagents can provide greater signal to noise ratios compared to those obtained with SA-HRP detection.

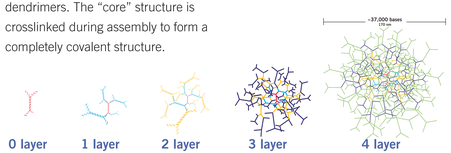
Dendrimer Components

Dendrimers are made from 7 single stranded DNAs. The strands are hybridized pair-wise to produced building block units called monomers.



Dendrimer Technology

Monomers are hybridized together to assemble various size DNA dendrimers. The "core" structure is crosslinked during assembly to form a completely covalent structure.

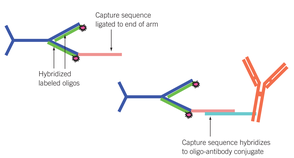


Methods

Preparation of UltraAmp™ reagents

Enzyme and Antibody labeling

Oligonucleotides are conjugated with HRP and anti-biotin antibodies. The sequences of these oligonucleotides are complementary with sequences on the outer arms of the dendrimer. These oligonucleotide-conjugates hybridize to the dendrimer when incubated for 30 minutes at room temperature in a specific buffer. This hybridization results in a dendrimer that has both HRP labels and anti-biotin antibodies on its outer arms.



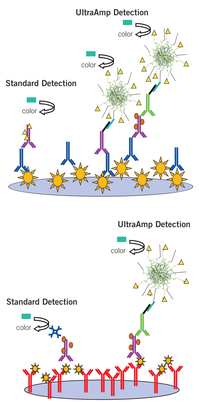
ELISA Assays

Indirect ELISA

- Antigen-coated plates from various manufacturers were used to test indirect ELISAs.
- For UltraAmp detection, incubation with the HRP labeled anti-human antibody was substituted by anti-human UltraAmp HRP or biotinylated anti-human antibody.
- For detection with biotinylated anti-human antibodies, unbound antibodies were washed off and detected with anti-biotin UltraAmp HRP

Sandwich ELISA

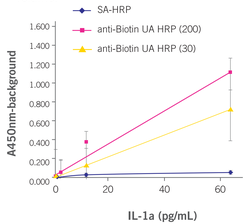
- Antibody pre-coated plates from various manufactures were used to test sandwich ELISAs.
- For UltraAmp detection, streptavidin-HRP was substituted by incubation with anti-biotin UltraAmp HRP



Results

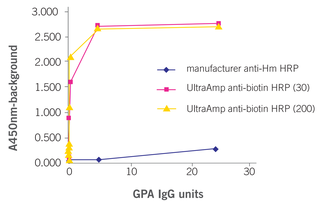
Sandwich ELISA for detection of IL-1a

- Comparison of signal response from anti-biotin UltraAmp HRP (30) and (200) compared to signal from streptavidin HRP. 18 ng of UltraAmp reagent were used for the detection in a 50 µL volume.



Detection of gastric parietal cell (H+ / K+) ATPase antibodies

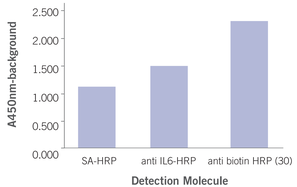
The presence of gastric parietal cell (H+/K+) ATPase antibodies along with clinical findings can be used in the diagnosis of pernicious anemia, which is characterized by elevated levels of these antibodies. Here we compared the detection of these Abs by HRP-labeled anti-human Abs (blue), or biotinylated anti-human followed by anti-biotin UltraAmp reagents with 30 (pink) or 200 HRPs (yellow).



Anti IL-6 vs anti-biotin

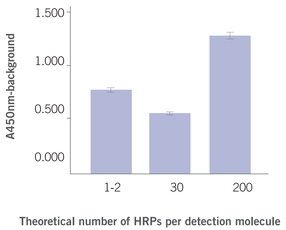
Detection of 150 pg/mL of IL6 in a sandwich ELISA.
Three detection schemes were compared:

1. Biotinylated anti IL6 followed by SA-HRP
2. HRP-labeled anti IL6
3. Biotinylated anti IL6 followed by anti-biotin HRP (30)



Amplification vs Theoretical HRP number

Detection of 37.5 pg/mL of IL-6 in a sandwich ELISA:
Comparison of three different detection molecules according to their HRP theoretical number



Alzheimer's Disease (AD)

The beta amyloid peptide is the major component in the extracellular plaques present in the brain of AD patients. In this experiment a monoclonal antibody captures the peptide, which is then detected by a rabbit antibody specific for the COOH-terminus of the peptide. The Rb antibody is then detected by HRP-labeled anti-Rb (blue) or biotinylated anti-Rb followed by anti-biotin UltraAmp HRP (pink)

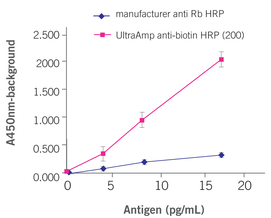


Table 1. Amplification achieved by UltraAmp in the detection of a variety of haptens

ELISA	Kit/Ab pair	Highest Amplification
Indirect	H pylori	10
Indirect	Histone	7
Indirect	GPA	218
Indirect	ACA	17
Indirect	B2GPI	30
Sandwich	IL-1a	80
Sandwich	IL-6	15
Sandwich	TNF	3

Conclusions

- **An UltraAmp reagent labeled with 200 HRPs usually yields greater signal than one labeled with 30 HRPs, but amplification is not proportional to the increase in the number of HRPs. In some cases, the output signal is very similar between the two reagents.**
- **Antibody stacking results in greater signal amplification.**
  - **There is a great improvement in signal when human antibodies are detected first with anti-human biotinylated followed by anti-biotin HRP labeled UltraAmp**
- **Anti-biotin HRP UltraAmp reagents provided up to 200-fold signal amplification in a variety of sandwich and indirect ELISAs**