Bio-plex 200 system

Bio-Plex Workstation Components

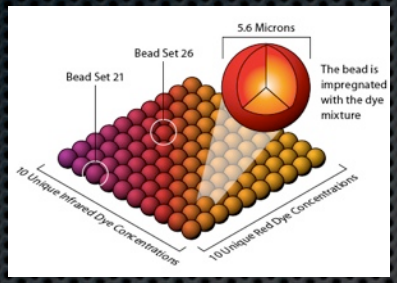
1. Array reader
2. Microplate platform
3. High-throughput fluidics (HTF)
4. Bio-Plex Manager software
5. PC station

Bio-plex 200 system was acquired through MOBITAG project (FP7-REGPOT-2008-1, GA 229518)

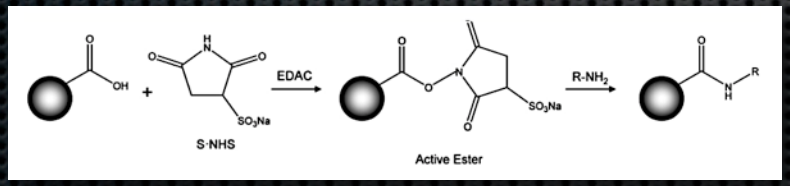
Location of the system: Department of Plant Virology
Institute of Plant Molecular Biology
Biology Centre of the Academy of Sciences
of the Czech Republic, v. v. i.

Principle of the technology*,**

An assay is performed on the surface of a 5.5 μm polystyrene bead. The beads are filled with different ratios of two different fluorescent dyes, resulting in an set of 100 distinct spectral address. Each set of beads are kept separately and coupled to unique specific ligand.

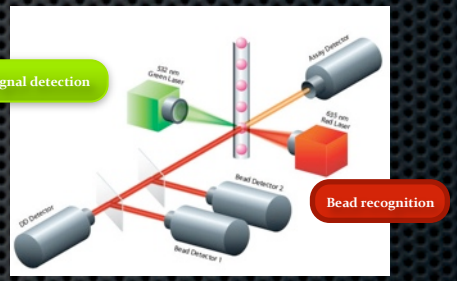


The procedure of coupling includes a two-step carbodiimide reaction that results in forming of stable ester. The deriver ester reacts with the primary amines (NH_2 groups) of proteins or amine-modified oligonucleotides to form a covalent bond.



Therefore, there are several possible types of assays. The choice depends on species planned to detect (proteins, nucleic acids, or immunoglobulins). By combining different sets of coupled beads in each single well, the necessary multiplexing is achieved.

While capturing the target specimen (depends on a kind of assay), each microbead acquires fluorescence, that is excites by green reporter laser. At the same time, the microbead is being classified using exciting by red classification laser. As the result, one data-point is generated – microbead classification number and associated fluorescence signal.



Alternatively Functionalized Beads

Functionality	Complementary Functionality	Example Biomolecule	Application	Utility
Antibodies	Antigens	Proteins, Peptides	Immunoassays, Enzyme activity assays	Expression analysis, Detection, Diagnosis
Antigens	Antibodies	Proteins, Peptides		Diagnosis, Serology, Activity determination
Oligonucleotides	Complementary Sequences	Oligonucleotides, PCR Amplicons	Nucleic acid assays	Expression analysis, Detection, Diagnosis
Avidin – Low Capacity	Biotin – conjugated Molecules	Proteins, Peptides, Oligonucleotides	Immunoassays, Nucleic acid assays, Receptor ligand assays, Enzyme activity assays	Expression analysis, Detection, Diagnosis, Peptide screening, Synthetic or recombinant antibody screening
Avidin – High Capacity	Biotin – conjugated Molecules	Proteins, Peptides, Oligonucleotides		
Hydrazide	Carboxyl Groups, Aldehyde Groups	Proteins, Peptides, Glyco-proteins, Carbohydrates	Immunoassays, Receptor ligand assays	Peptide screening, Synthetic or recombinant antibody screening
Maleimide	Sulfhydryl Groups	Proteins, Peptides		
Species – specific Anti-Immunoglobulin	Antigen – specific Antibody	Restricted Availability Antibodies	Immunoassays	Antibody screening, Vaccine efficacy testing

* More information on Bio-Rad web-pages, following direction: Life Science Research > Multiplex Suspension Array System > Bio-Plex Instruments and Software > Bio-Plex 200 System
** Images were taken from www.bio-rad.com, www.radixbiosolutions.com

Scheme of microsphere-based DNA hybridization

PCR amplification step (utilizes biotin-dUTP) — 1 h

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Hybridization steps — 1.5-2 h

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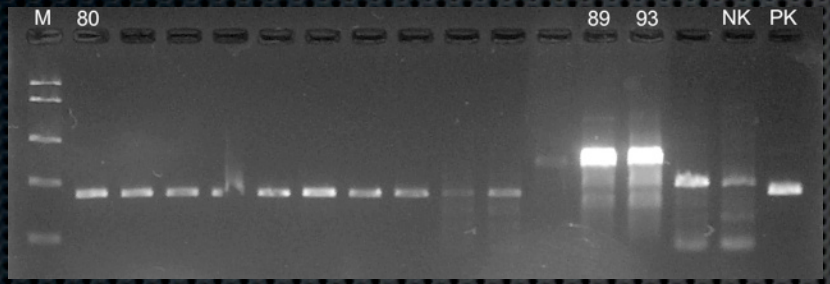
Data acquisition and processing — 30 min

Total time: up to 4 hours, including all procedures

Comparison of results obtained with:

(I) conventional PCR



Phoresis of PCR amplification of a CaMV genome region using total DNA isolated from plant samples

(II) direct DNA hybridization assay evaluated using Bioplex 200