Streamlined Bead-Based Assays for the High-Throughput Analysis of Antibodies and Proteins

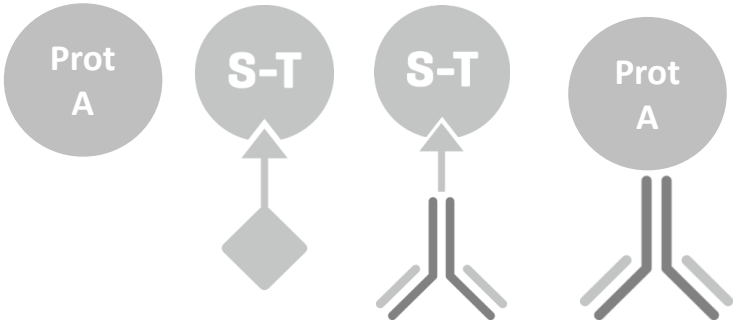
5th Annual SLAS Conference 2016
San Diego

PAIA assays in a nutshell

- No-wash bead-based assays with fluorescence detection
- Sample volume < 10 µL per well
- High throughput in 384-well plates
- < 60 min/plate, can be enhanced by incubation in parallel
- Incubation, bead separation and detection in the same plate
- Runs on standard fluorescence readers
- Applications: Quantification of proteins and antibodies and binding screens
- Assays kits contain PAIA*plates* with dried capture beads and a ready-to-use buffer with fluorescence marker for a specific analyte

Assay principle

We use functionalized capture beads



to bind analytes from the sample (e.g. antibodies, proteins)

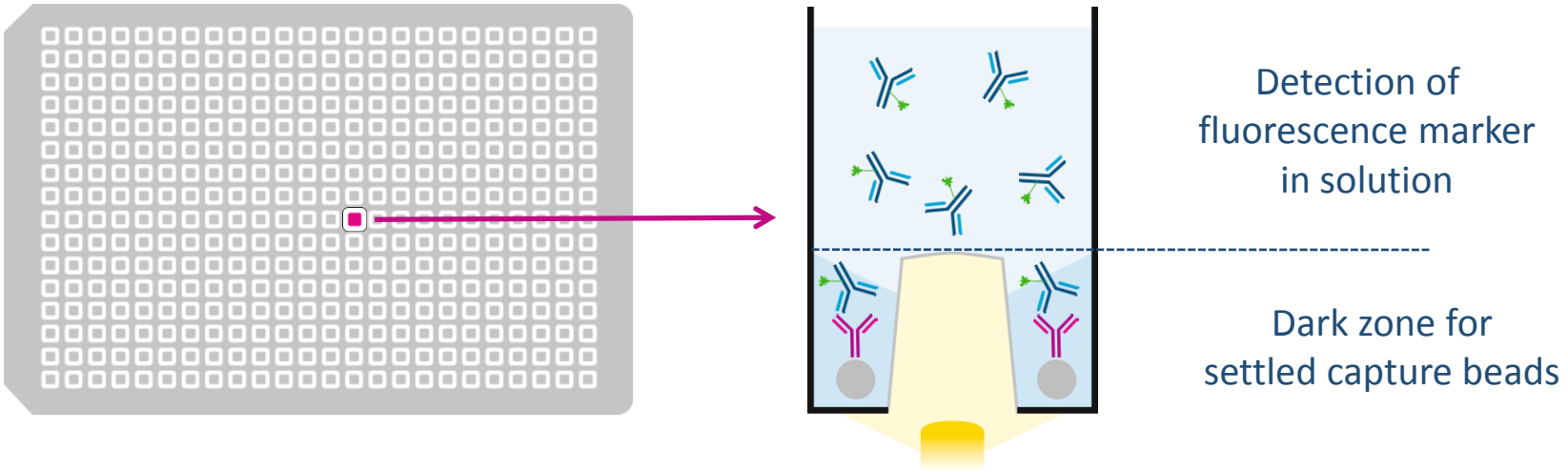
and detect the binding using fluorescence markers

Our innovation

- We exploit the advantages of bead based assays
- We measure the fluorescence intensity in solution (not on the beads)
- We use one microplate for incubation, bead separation & detection

Features of the PAIA*plate*

- 384-well plate with standard dimensions
- Black bottom for blocking excitation and emisson of settled beads
- Transparent protrusion to direct bead settling to the well bottom and enabling fluorescence detection of unbound markers in solution



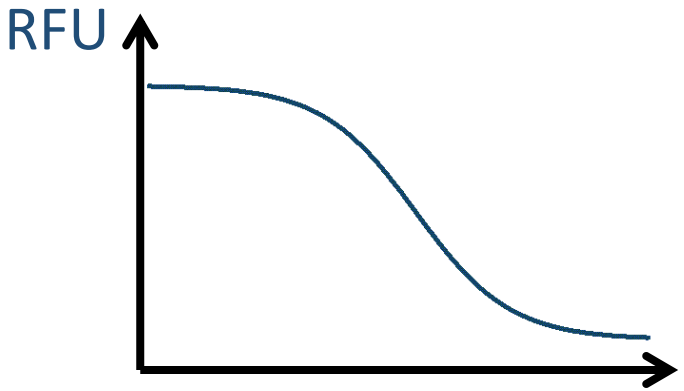
Generic assay formats

Direct assays (analyte binds marker to the bead)

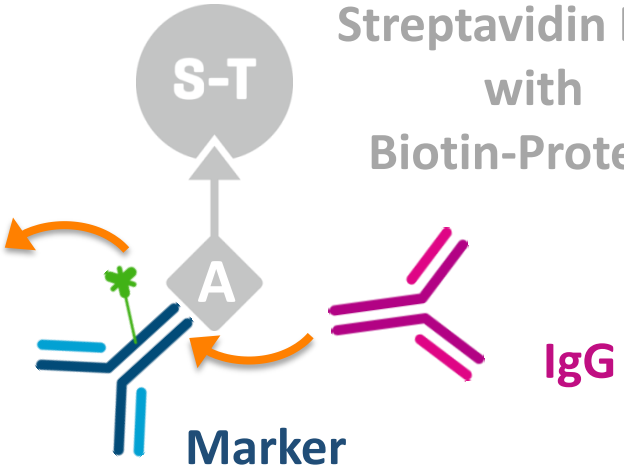
Anti-F(ab) marker

IgG

Protein A Bead

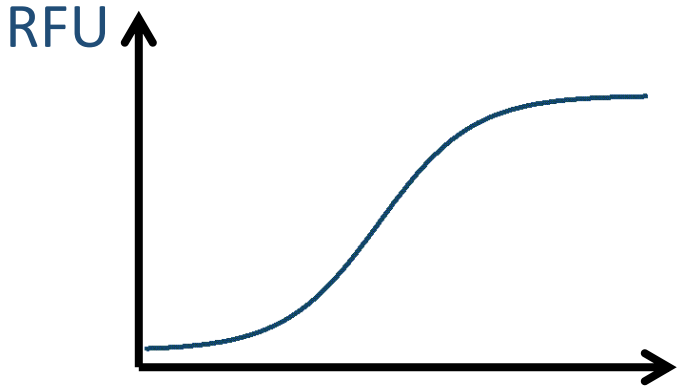


Competitive assays (analyte replaces marker from the bead)



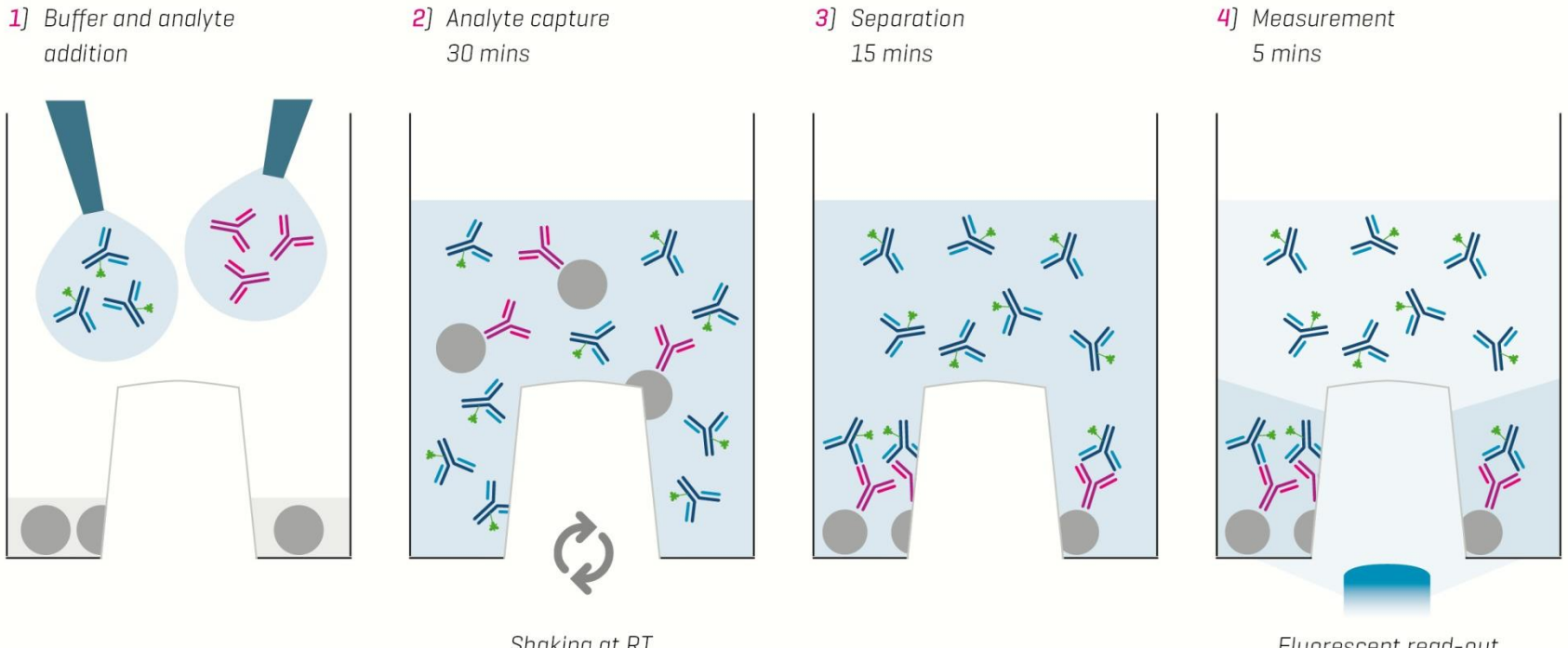
Streptavidin Bead with Biotin-ProteinA

IgG



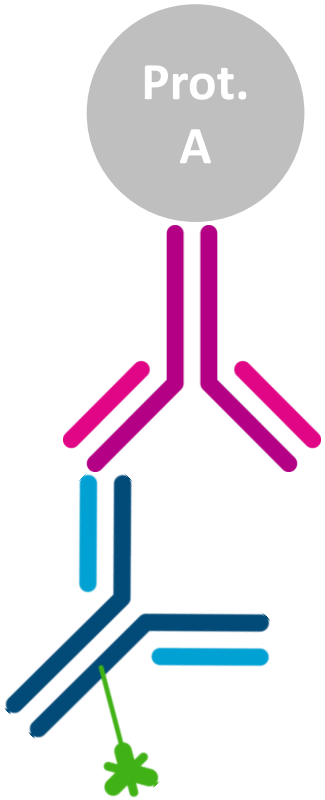
Assay workflow

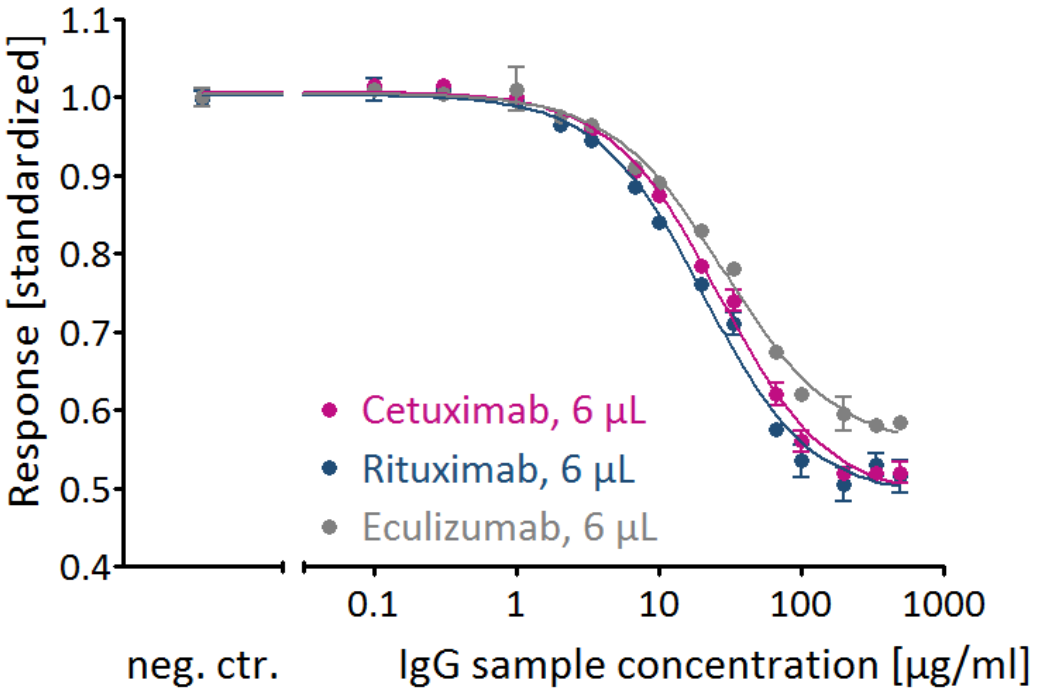
- Starts with PAIA*plate* containing pre-dispensed dried capture beads
- Add 50µL ready-to-use buffer with marker into the wells you want to use
- Add 2-10 µL of sample containing the analyte
- Incubate the plate on orbital shaker for 30 mins, then let beads settle 15 mins.
- Measure on a fluorescence plate reader or microscope (bottom read).



Human IgG quantification: early clone screening

- Direct assay format
- Capture via ProteinA beads, detection with human IgG Fab-specific marker





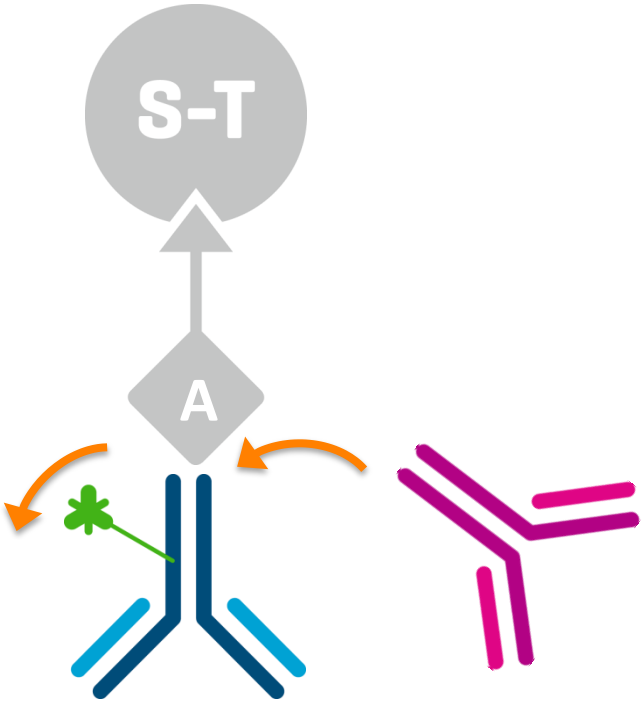
Pilot study early clone selection (IgG1)

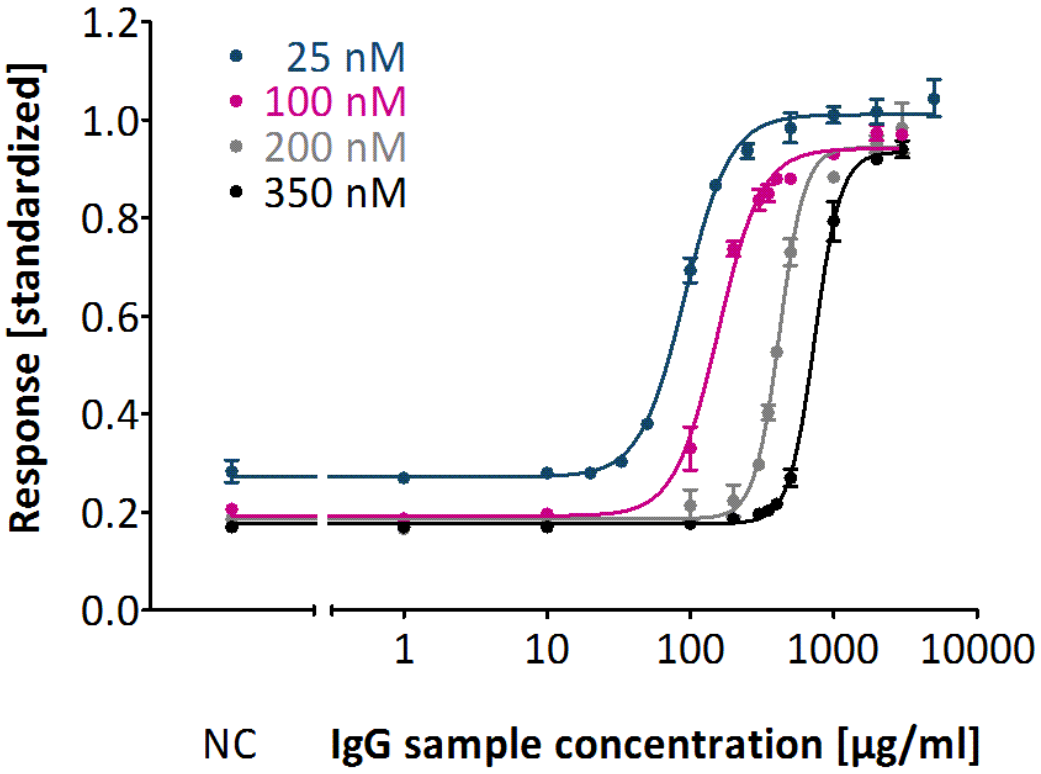
- Cell culture supernatants from CHO cells 10 days after seeding
- Four 96-well plates combined onto one PAIAplate
- Four replicate PAIAplates tested in parallel on four shakers
- All plates (1536 samples and standards) run and analyzed in 2,5 hours
- 169 of 192 top clones (88%) identical with Octet384 ranking
- Identical results for each PAIAplate
- Calibration can be done on a separate plate

	plate 1				plate 2				plate 3				plate 4			
Calibration	**1**	2	3	4	1	**2**	3	4	1	2	**3**	4	1	2	3	**4**
	9	8	6	1	9	**8**	6	1	3	3	**2**	0	4	4	2	**0**
	25	26	22	18	29	**30**	26	23	23	24	**20**	16	29	31	26	**24**
	8	7	5	1	11	**10**	8	3	6	5	**4**	0	8	7	6	**1**
	66	76	69	77	77	**89**	83	94	69	79	**72**	81	83	96	91	**104**
	52	59	52	56	63	**72**	65	73	58	66	**59**	65	58	65	58	**64**
	37	40	34	34	40	**44**	38	39	34	37	**31**	30	39	43	37	**38**
	12	11	9	4	16	**15**	12	8	13	12	**10**	5	13	13	10	**5**
	113	129	132	149	102	**118**	117	133	100	115	**114**	130	96	111	109	**124**
	105	121	121	138	101	**117**	116	132	99	114	**112**	128	97	112	109	**125**
	10	10	8	3	10	**9**	7	2	9	8	**7**	2	13	12	10	**5**
	102	117	116	132	118	**135**	139	157	103	119	**118**	134	122	139	145	**163**
	59	67	60	66	59	**67**	60	66	64	73	**66**	74	66	75	69	**77**
	117	134	138	156	115	**131**	135	152	106	122	**122**	139	99	114	113	**129**
	140	158	172	191	167	**183**	215	230	145	163	**180**	198	146	163	181	**198**
	64	73	66	74	65	**75**	68	76	57	64	**57**	63	50	57	50	**53**
	117	134	138	156	96	**111**	108	124	85	98	**93**	106	95	110	108	**123**
	10	10	8	3	12	**11**	9	4	8	8	**6**	1	13	13	10	**5**
	12	12	10	4	16	**15**	13	8	12	11	**9**	4	17	17	14	**9**
	0	0	0	0	0	**0**	0	0	0	0	**0**	0	0	1	0	**0**
	15	15	12	7	17	**17**	14	9	14	14	**11**	6	18	18	15	**11**
	118	135	139	157	160	**176**	203	219	124	141	**148**	167	130	147	157	**175**
	64	74	67	74	57	**65**	58	64	48	53	**47**	50	65	75	68	**76**
	84	97	92	105	94	**108**	105	120	89	103	**99**	113	87	100	96	**109**
	8	8	6	1	12	**11**	9	4	8	7	**6**	1	11	11	8	**3**
	130	147	156	175	168	**184**	216	230	114	130	**133**	151	128	145	153	**172**
	77	89	83	94	88	**102**	97	111	72	83	**76**	86	87	101	96	**110**

IgG quantification for bioprocess development

- Streptavidin capture beads with biotinylated Protein A
- Fluorescence marker: labeled human IgG
- Adjustment of dynamic range by changing the binding capacity of capture beads

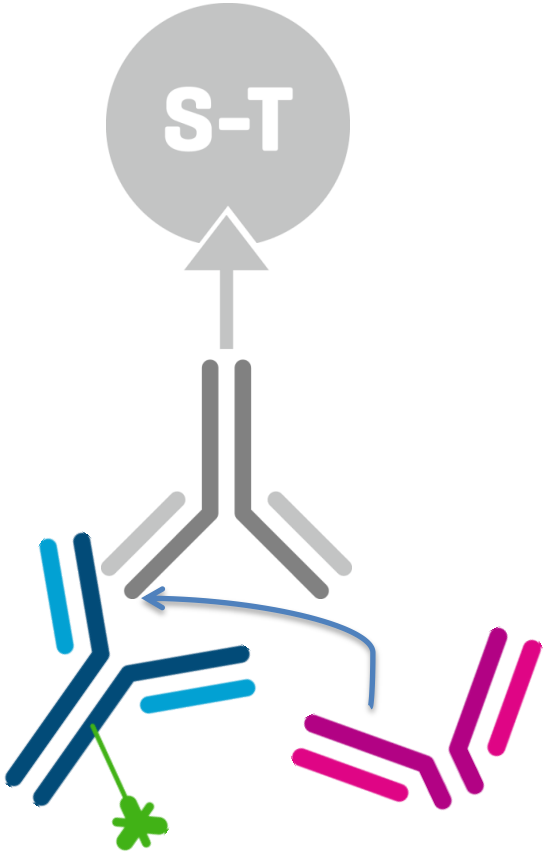


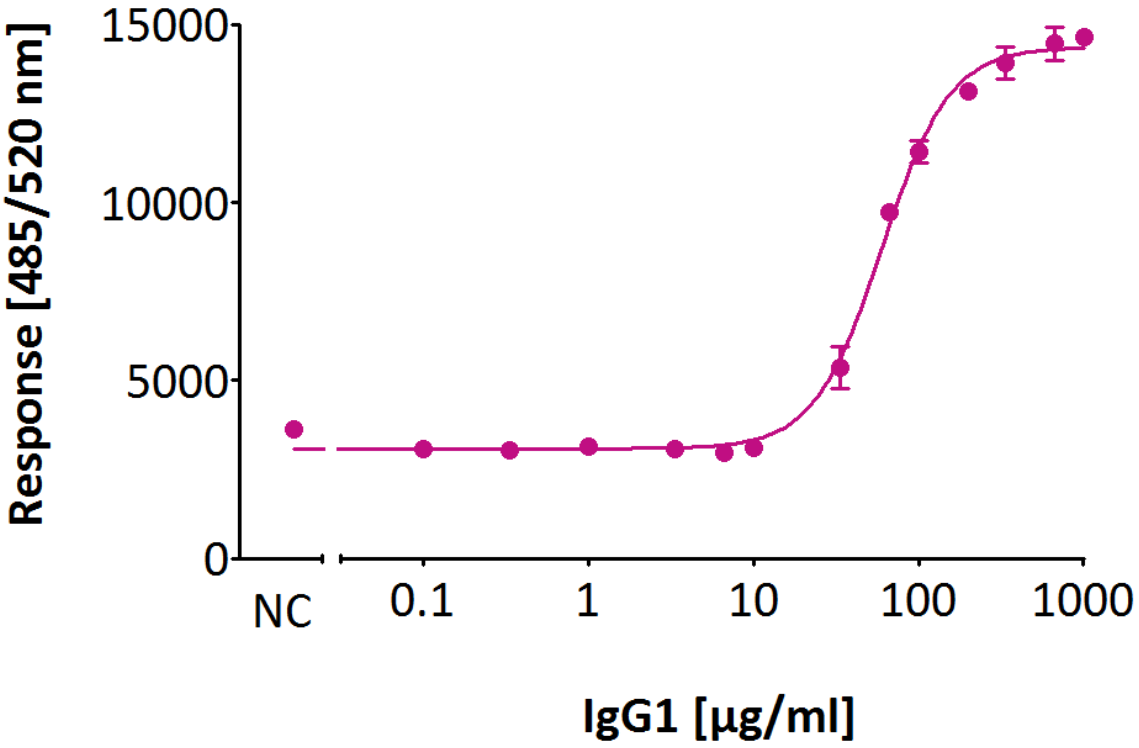


Fab Assay: CH1 Capture

- Competitive assay format
- Capture via bead-coupled anti human CH1 antibody

beta

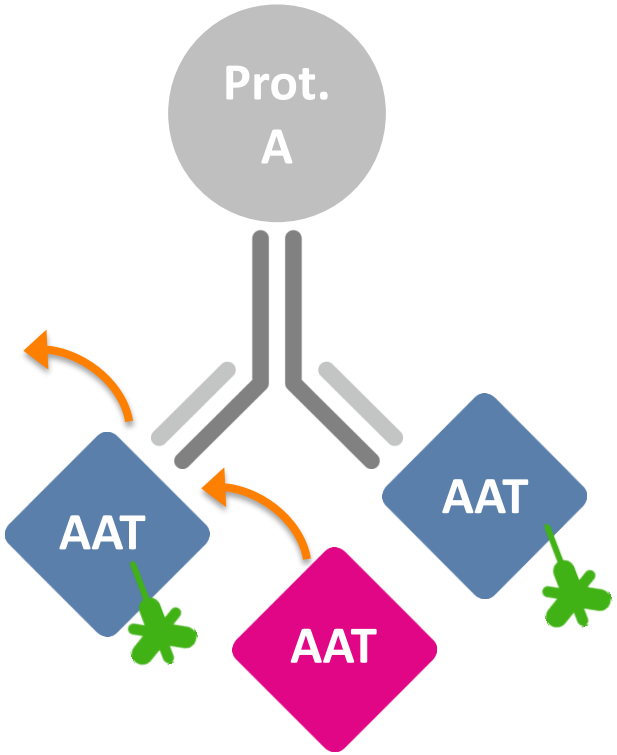


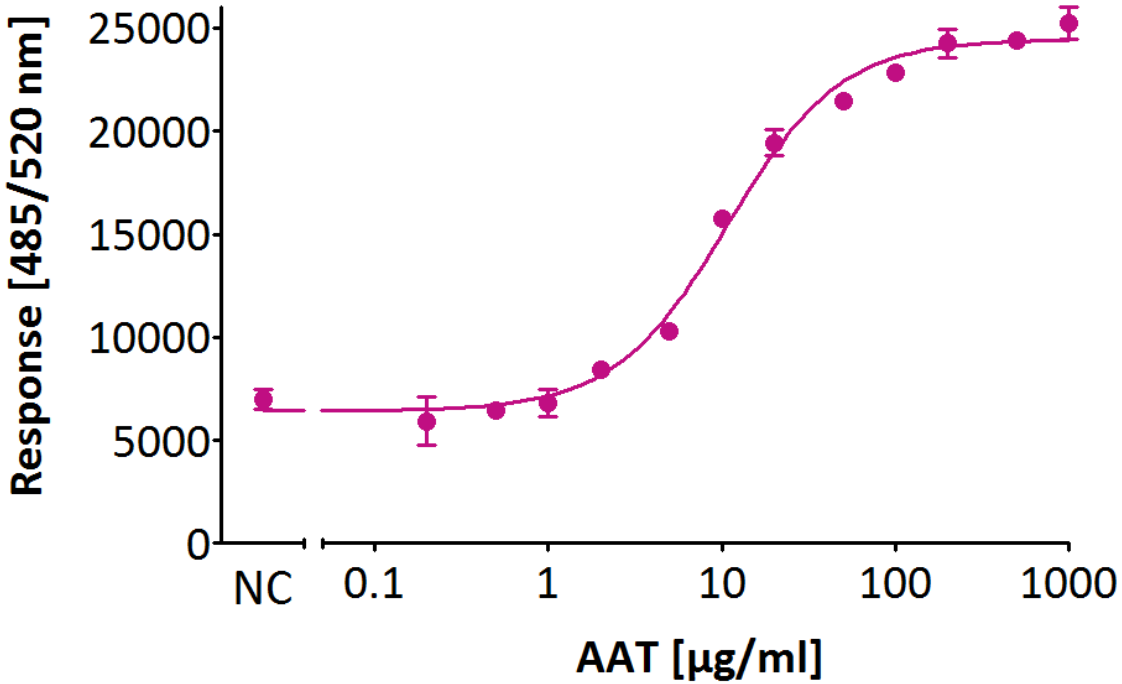


Custom Assay: human α–Antitrypsin (AAT)

- Competitive assay format
- Capture via bead-coupled rabbit anti-AAT antibody

custom

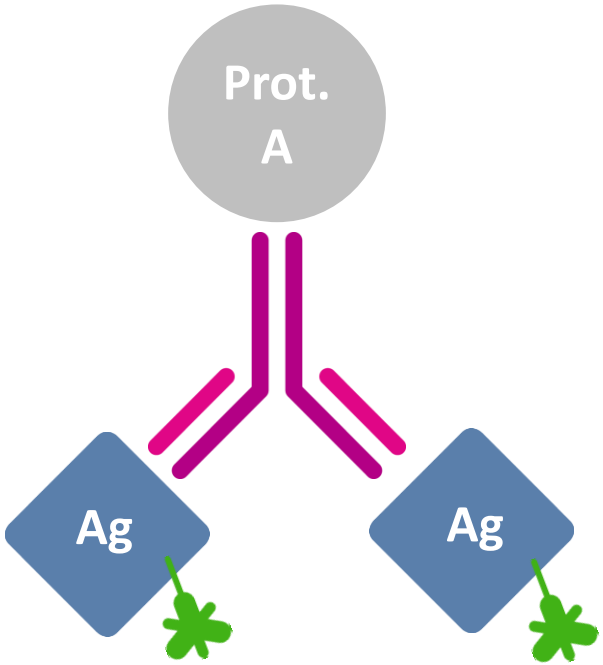


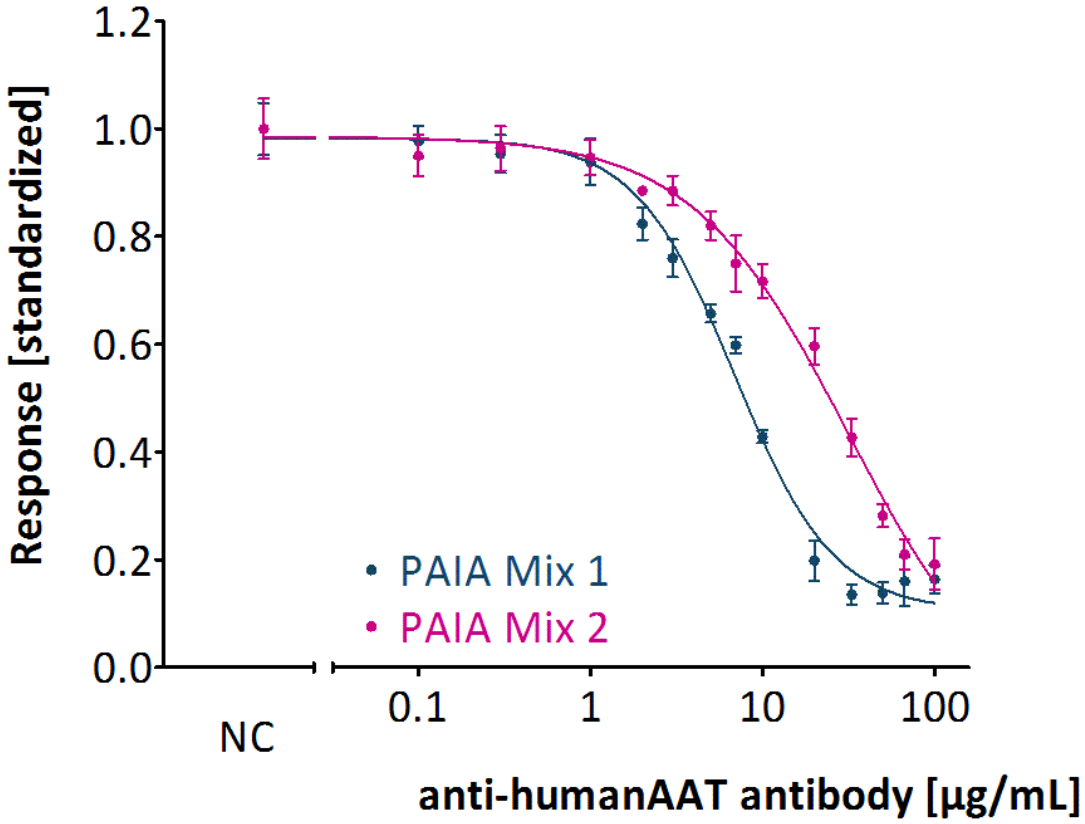


Antibody screening assay

- Capture Beads: Protein A
- Fluorescence marker: labeled antigen (Ag) here: AAT

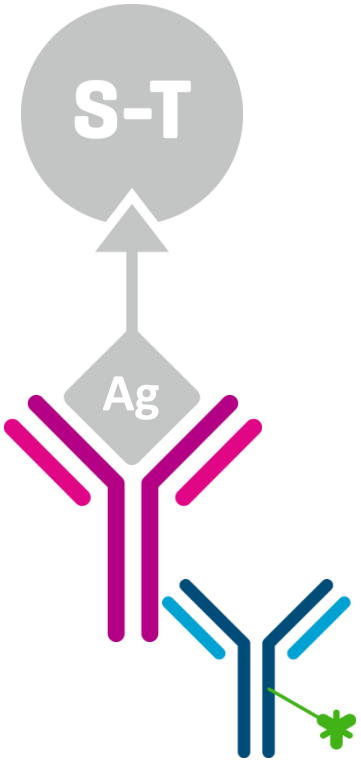
custom



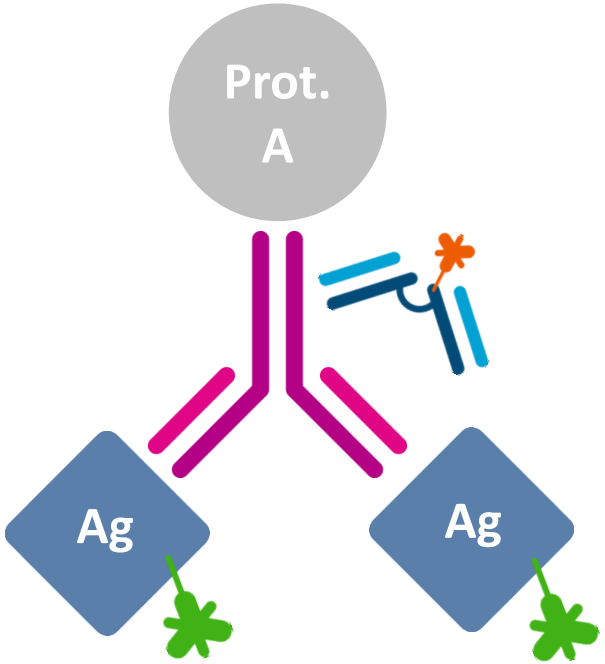


Antibody screening assay formats

- Capture Beads: Streptavidin with biotinylated antigen
- Fluorescence marker: labeled IgG anti-Fc specific marker



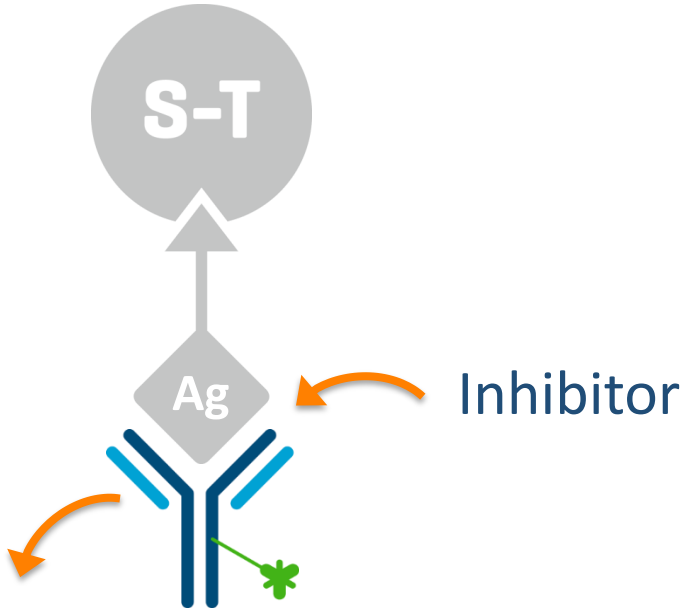
- Capture Beads: ProteinA
- Fluorescence marker: labeled antigen, optional second IgG anti-Fc specific marker



Two colour assay

Inhibition screening assays

- Capture Beads: Streptavidin with biotinylated antigen/target
- Fluorescence marker: labeled antibody or target binding protein



Inhibition generates high fluorescence signals

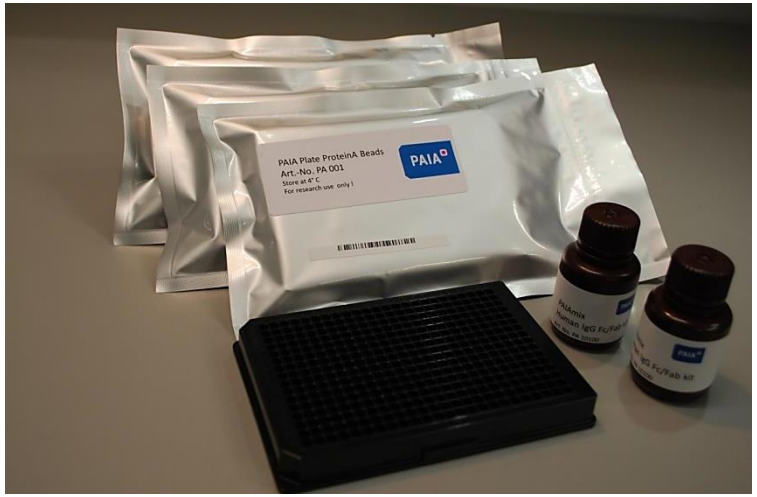
Take Home Message

- Streamlined bead-based immunoassay platform with
- High throughput and simple, automation-friendly workflow
- No washing, no blocking, no sensor regeneration
- Sample volumes: < 10 µL
- Fluorescence reader or fluorescence microscope (e.g. Cellavista)
- Straightforward data analysis (PAIA standalone or reader software modules)

Time saving, usually capex-free and robust tool for screening applications

The Company

- Founded in 2014, privately owned and funded
- Research & technology development since early 2013
- Patent pending platform technology
- Based in Cologne, Germany
- Products: assay kits and custom assay development



Thank you!

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