# Quantibody® Technical Notes

Quantibody® Technology Overview:

Enzyme-Linked ImmunoSorbent Assay (ELISA) has been routinely used in the lab for cytokine detection and quantification for over 40 years. In this method, target protein is first immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection is accomplished by incubating this enzyme-complex with a substrate that produces a detectable signal. Within a certain range, the signal intensity is proportional to the target protein concentration, which can be used for protein quantification through parallel internal standard controls. If the target protein is captured through binding to its immobilized specific capture antibody, a sandwich ELISA assay is performed. Because sandwich ELISA uses a pair of protein specific antibodies, it greatly increases the assay detection specificity and sensitivity.
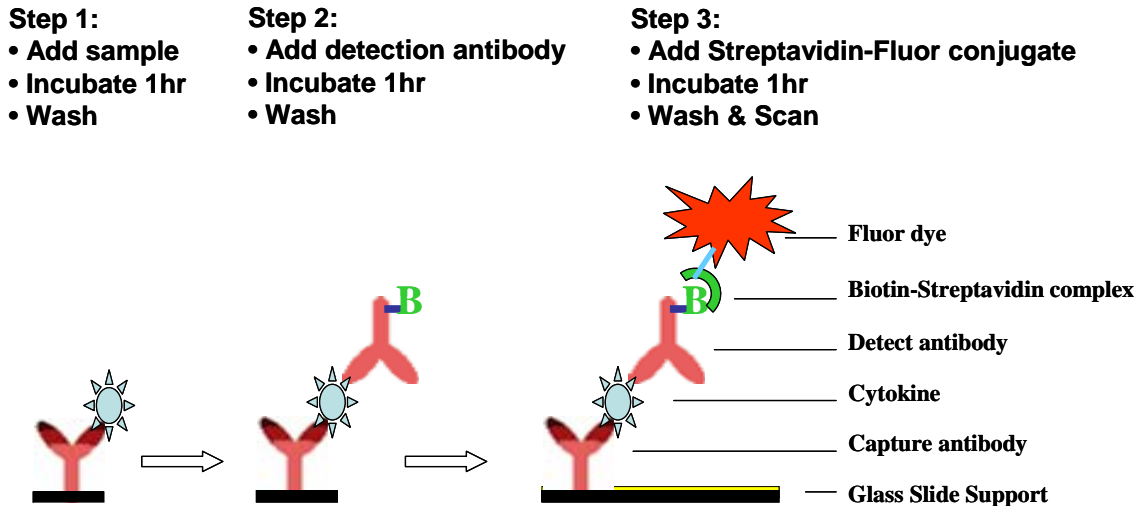
Quantibody® array is an array-based multiplex sandwich ELISA system for simultaneous, quantitative measurement the concentration of multiple proteins in serum, plasma, CSF, cell lysate, conditioned media and other body fluid. It combines the advantages of the high detection specificity / sensitivity of ELISA and the high throughput of the arrays.

Capture antibodies

One standard glass slide is spotted with 16 identical arrays of capture antibodies
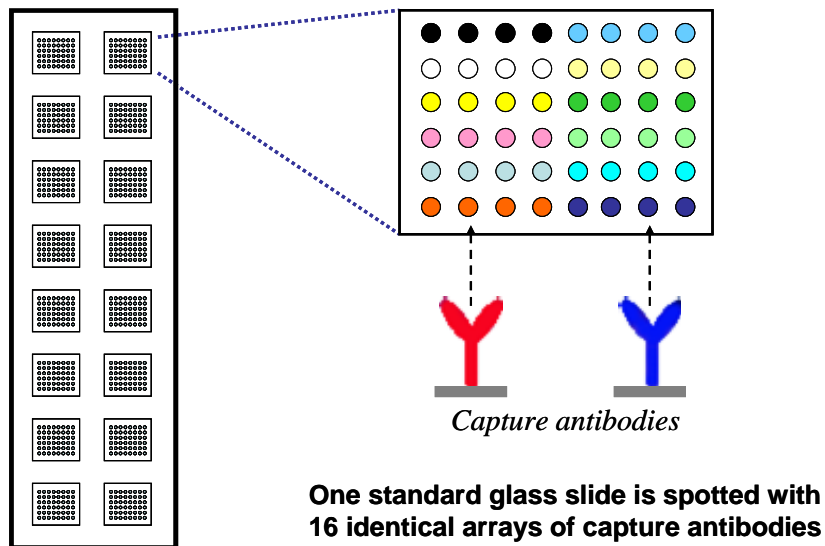
Like a traditional sandwich-based ELISA, it uses a pair of antigen-specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target protein is trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different epitope of the target cytokine. The protein-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled fluorescence dye using a laser scanner.

Unlike the traditional ELISA, Quantibody products use an array format. By arraying multiple capture antibodies onto a glass support, multiplex detection of proteins in one experiment is made possible. In detail, one standard glass slide is spotted with 16 wells of identical antibody arrays. (See Figure 1). Each antibody, together with the positive and negative control is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples in one slide. Four slide chips can be nested into a tray, which matches a standard microplate and allows for automated robotic high-throughput process of 64 arrays simultaneously. For protein

quantification, the reagent kit includes protein standards, whose concentration has been predetermined, were provided to generate a six-point standard curve of each protein. Standards and samples are assayed simultaneously. By comparing signals from unknown samples to the standard curve, the unknown protein concentrations in the samples are determined.

Step 1:
• **Add sample**
• **Incubate 1hr**
• **Wash**

Step 2:
• **Add detection antibody**
• **Incubate 1hr**
• **Wash**

Step 3:
• **Add Streptavidin-Fluor conjugate**
• **Incubate 1hr**
• **Wash & Scan**

— Fluor dye

— Biotin-Streptavidin complex

— Detect antibody

— Cytokine

— Capture antibody

— Glass Slide Support

Quantibody® arrays enable a researcher accurately determine the concentration of multiple proteins simultaneously and inexpensively. Our current Quantibody® Human Cytokine Antibody Array 2000 enables scientists to quantitatively detect 120 cytokines in a single experiment. Compared to the traditional ELISAs, which requires large sample volumes and significant processing time, now you can get 480 times more data in as little as 100 µl samples in a single day.

Quantibody® Product Highlights:
- More Data: Detect up to 40 cytokine concentrations in a single array
- Less sample: Requires as little as 50 µl of valuable sample
- Save time: Get the results the same day (approx. 4-hour processing time)
- High throughput: Hundreds of samples can be processed by one technician in a single day.
- Affordable and cost effective (Less than $0.8 per data point)
- Diverse sample types: serum, plasma, BAL, CSF, conditioned media and other body fluid.
- High sensitivity: Similar or better detection sensitivity as ELISA
- High specificity and system reproducibility
- Flexibility: User can choose own panels from over 300 target proteins
- Signal stability: The fluorescence signal on slides can retain for years
- Simplicity: Q-Analyzer software for 1 step data computation.
- Testing service available. You name the markers, we return the results.

Quantibody® Products Performance:

1. ELISA sensitivity:

**Quantibody has similar detection sensitivity as ELISA**
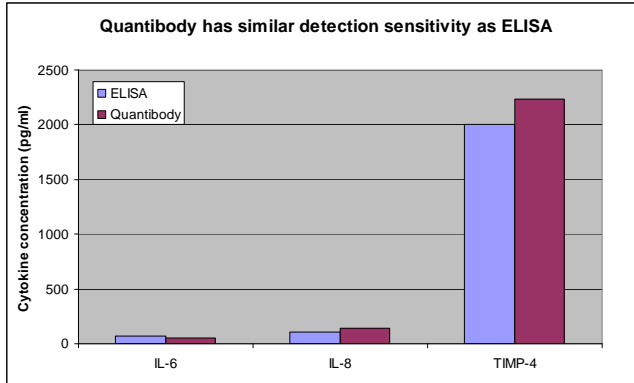
The detection sensitivity of a specific cytokine in the **Quantibody®** array was evaluated by comparing to its single cytokine ELISAs. To ensure data quality, the same pairs of antibodies together with the same antigen source were used, and both ELISA and **Quantibody®** protocols were optimized for sensitivity and specificity. Both systems were then used to determine the IL-6, IL-8, and TIMP-4 protein concentrations in the same human CSF sample. The calculated concentration of all the three cytokines turned out to be highly comparable, indicating that **Quantibody® arrays** have similar detection sensitivity compared with ELISAs. (Figure 2)

2. Stability & Reproducibility:

In order to evaluate the stability and reproducibility of the Quantibody platform, six Quantibody human TH1/TH2 kits developed over time (ranging from 1 to 20 months old) were used simultaneously to determine the cytokine concentration in the same sample. (Figure 3). After parallel processing of the experiments, the slides were then scanned using the same parameters. The extracted fluorescence signals of the 6-point standards

from these six slides for the same cytokine turned out to be highly comparable as illustrated by IL-6. Meanwhile, the sample cytokine concentrations were then calculated separately with Q-Analyzer Software by these six kits. The average inter-assay CV of 10 cytokines by the six different kits is about 12% (Table 1). Our data proves that **Quantibody®** arrays can be reliably used for protein quantification.

Product Stability Test (IL-6)
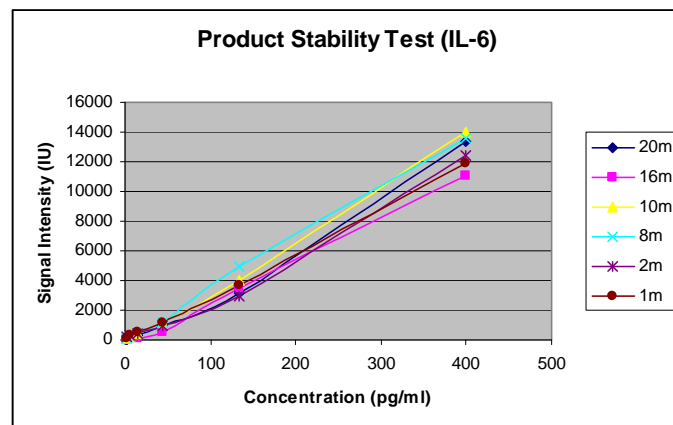
Cytokine	6 different slides (month)						Ave	Std Dev	Inter-assay CV
(pg/ml)	20m	16m	10m	8m	2m	1m			
IL-2	412.6	324.1	437.5	381.5	413.1	414.2	397.2	40.0	10.1%
IL-4	566.6	552.3	539.6	543.8	592.4	543.8	556.4	20.1	3.6%
IL-5	453.1	432.3	390.9	390.6	418.0	510.5	432.6	45.2	10.5%
IL-6	231.5	233.5	184.8	179.3	180.8	205.6	202.6	25.1	12.4%
IL-8	248.9	222.2	212.5	227.8	211.6	204.3	221.2	15.9	7.2%
IL-10	255.9	224.0	190.4	293.1	264.7	210.0	239.7	38.2	15.9%
IL-13	210.2	254.0	185.8	213.8	216.4	223.9	217.4	22.1	10.2%
GM-CSF	242.9	194.6	211.8	194.6	224.1	227.2	215.9	19.2	8.9%
IFNg	513.6	488.9	399.8	372.1	453.5	413.9	440.3	54.6	12.4%
TNFa	397.8	317.7	428.8	319.0	413.9	363.9	373.5	47.9	12.8%

3. System CV (Coefficient of Variation)

Quantibody human inflammation array 1 kits were used to test the system CV (Table 2). Four wells of the same sample were run in parallel with the standards by two distinct researchers in two different occasions. The average Intra-assay CV is found to be 6.6% by researcher 1 (RA1) and 10.9% by researcher 2 (RA2). The average Inter-assay CV is 12.4%.

RA1	SA1				SA2				Average		Stdev		Intra-CV	
ID	#1	#2	#3	#4	#5	#6	#7	#8	SA1	SA2	SA1	SA2	SA1	SA2
IL-1a	231	230	224	234	20	22	22	20	230	21	4	1	1.7%	5.3%
IL-1b	625	611	652	696	153	165	171	165	646	163	37	8	5.8%	4.6%
IL-4	902	918	905	992	310	296	321	298	929	306	43	12	4.6%	3.9%
IL-6	612	529	545	602	153	119	152	121	572	136	41	19	7.2%	14.0%
IL-8	411	480	423	464	233	273	255	255	445	254	33	17	7.4%	6.5%
IL-10	601	544	557	591	146	127	115	121	574	127	27	14	4.7%	10.8%
IL-13	535	582	512	511	234	213	224	258	535	232	33	20	6.2%	8.4%
MCP-1	1073	1017	943	944	440	477	497	433	994	462	63	30	6.3%	6.6%
IFNg	489	434	401	422	126	142	132	124	436	131	37	8	8.6%	6.4%
TNFa	590	592	614	519	115	100	114	116	579	111	42	7	7.2%	6.6%

RA2	SA1				SA2				Average		SD		Intra-CV	
ID	#1	#2	#3	#4	#5	#6	#7	#8	SA1	SA2	SA1	SA2	SA1	SA2
IL-1a	184	185	225	149	19	27	29	21	186	24	31	5	16.6%	20.3%
IL-1b	521	439	487	648	130	125	145	164	524	141	90	18	17.1%	12.4%
IL-4	864	846	840	804	229	210	268	287	838	248	25	35	3.0%	14.3%
IL-6	326	356	426	461	89	88	94	88	393	89	62	3	15.8%	3.2%
IL-8	525	462	470	538	225	214	232	264	499	234	38	22	7.7%	9.2%
IL-10	639	607	648	702	142	202	141	138	649	156	40	31	6.1%	19.9%
IL-13	484	449	519	589	217	222	193	208	510	210	60	13	11.7%	6.1%
MCP-1	1045	968	970	1107	416	348	316	312	1023	348	67	48	6.5%	13.9%
IFNg	542	526	489	515	128	146	187	166	518	157	22	26	4.3%	16.3%
TNFa	594	562	600	618	154	175	162	142	593	158	23	14	3.9%	8.9%

4. LOD (Limit of Detection)

The lowest detection sensitivity of each protein which was listed in each premade Quantibody kit is defined as the corresponding concentration at two standard deviations above the median fluorescence of certain replicates of the negative control (0 pg/ml). Take IL-5 in certain Quantibody® array for example, the signal intensity for serial dilutions of IL-5 was listed in the following table. Now if we have 8 negative controls for IL-5 in the same array with the signal intensity reading of 15, 14, 20, 25, 20, 22, 15, and 20 respectively, then the average is 19 with the standard deviation of 4. The calculated concentration based upon above standard curve at the signal intensity of 27 (19 + 2*4) will be 1.1pg/ml. Then the LOD of IL-5 in this array is 1.1pg/ml.

IL-5	Control	Std1	Std2	Std3	Std4	Std5	Std6
Con (pg/ml)	0	2	8	31	125	500	2000
Signal (IU)	19	25	58	153	687	2334	14935

5. System Recovery.

The applicability of the Quantibody arrays in certain media was evaluated by the spiking recovery test. In such test, different levels of the recombinant proteins were spiked into the media. With the non-spiked media as negative control, the recovery rate for each cytokine was then determined by subtracting the endogenous cytokine level from the observed value and divided by the spiking cytokine concentration. Following is the recovery rate for Quantibody mouse cytokine array 1 in conditioned media (CM).

Cytokine	Spiking (pg/ml)	CM	CM+Ag	CM%
GM-CSF	1,000	47	868	82%
IFNg	40,000	0	52,982	132%
IL-1a	10,000	0	12,295	123%
IL-1b	40,000	218	49,953	124%
IL-2	5,000	0	3,927	79%
IL-3	5,000	0	3,823	76%
IL-4	500	0	482	96%
IL-5	5,000	0	5,102	102%
IL-6	1,500	34	1,453	95%
IL-9	50,000	0	49,153	98%
IL-10	5,000	0	5,448	109%
IL-12	25,000	0	22,182	89%
IL-13	20,000	0	26,127	131%
IL-17	2,000	0	2,265	113%
KC	20,000	0	19,984	100%
M-CSF	500	17	459	88%
RANTES	20,000	0	21,114	106%
TNFa	20,000	0	20,043	100%
VEGF	3,000	2,787	6,147	112%