

Development of anti-drug antibodies assay (Ligand Binding Assay)

Anti-drug antibodies (ADA) assay is crucial to application for biopharmaceuticals including antibody drugs. TRC offers a series of services to prepare critical reagents for ADA bridging assay, develop and validate the assay method, and conduct study sample analysis.

SULFO-TAG labeled drug & biotinylated drug

- A drug is reacted with each of NHS-Ester biotin and SULFO-TAG under alkaline conditions.
- Buffer exchanged and purified.

Bridging assay

- Optimum concentrations of SULFO-TAG labeled drug and biotinylated drug are determined.
- Serum concentration (dilution factor) is determined.

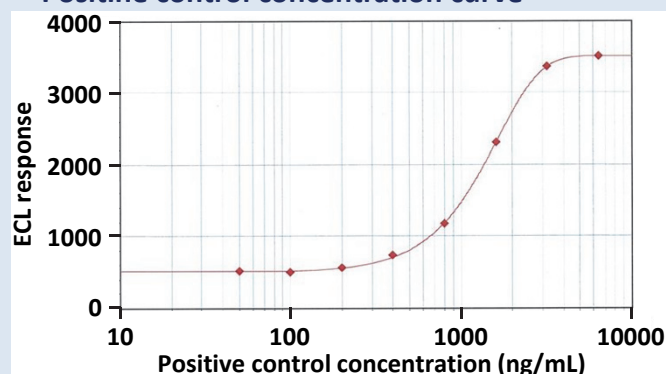
Evaluation of critical reagents

- Protein quantification (BCA assay, etc)
- SDS-PAGE
- Titering Assay ...etc.

Under Japanese Criteria
For Reliability of
Application Data

Development of ADA assay

● Positive control concentration curve



● Precision of ADA response

	Positive control concentration			
	NC (0 ng/mL)	LPC (300 ng/mL)	MPC (1000 ng/mL)	HPC (5000 ng/mL)
ECL response	371	726	1810	4063
	441	736	1789	4105
	485	740	1734	4083
Mean	432	734	1778	4084
%CV	13.3	1.0	2.2	0.5

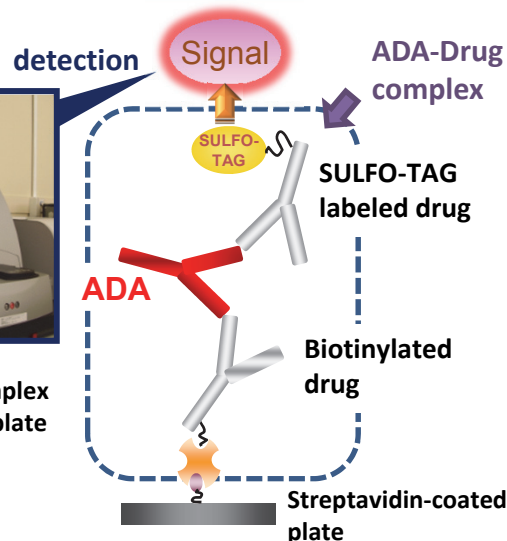
● Drug-spiked serum

Drug Concentration in serum sample (μg/mL)	Positive control concentration					
	NC (0 ng/mL)		LPC (300 ng/mL)		HPC (5000 ng/mL)	
	ECL response	%Inhibition	ECL response	%Inhibition	ECL response	%Inhibition
500	345	27.7	291	59.3	290	92.3
100	393	17.6	366	48.8	396	89.4
20	429	10.1	416	41.8	479	87.2
4	426	10.7	418	41.5	1201	67.9
0.8	413	13.4	462	35.4	3273	12.7
0.16	420	11.9	606	15.2	3597	4.0
0.032	466	2.3	709	0.8	3669	2.1
0	477	—	715	—	3747	—

ECL System



- (1) Prepare complex
- (2) Capture on plate
- (3) Detect



Calculation of cut point

Individual serum	Screening assay	Specificity confirmation assay	
	ECL response	ECL response	%Inhibition
No.1	627	509	18.82
No.2	461	436	5.42
No.3	583	501	14.07
No.4	597	420	29.65
No.5	620	589	5.00
No.6	616	542	12.01
No.7	515	406	21.17
No.8	505	394	21.98
No.9	490	486	0.82
No.10	651	528	18.89
Mean	567	—	14.78
SD	67	—	9.03
Cut point	677	—	42.67

Screening cut point : Mean + 1.645 × SD

Specificity cut point : Mean + 3.09 × SD

Outlier and normal distribution are assessed by JMP analysis software

Shapiro-Wilk W test

