Avidity ELISA

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Aim

To quantify the avidity of a polyclonal antiserum to a specific antigen using sodiumthiocyanate (NaSCN). The avidity-index is the NaSCN-concentration at which 50% of the bound antibodies is eluted off.

Materials

- Deepwell plates: TreffLab #96.8564.9.01
- ELISA-plates: Greiner Bio-one #655092
- Plastic seals: Nunc #236266
- Platereader: Bio-Rad Model 680 Microplate Reader
- Platewasher: ELx405 Auto Plate Washer
- PBS pH 7.4: Gibco #10010-015
- BSA (Bovine Serum Albumine) Fraction V, ≥ 96%; Sigma #9647
- Tween-20: Merck #8.22184.0500
- Blockingbuffer (BB): PBS + 0.05% v/v Tween-20 + 3% w/v BSA
  e.g. for 500 ml BB: 500 ml PBS + 250 µl Tween-20 + 15 g BSA
- Coatingbuffer (CB): PBS
- Dilutionbuffer (DB): PBS + 0.05% v/v Tween-20 + 0.5% w/v BSA
  e.g. 500 ml PBS + 250 µl Tween-20 + 3 g BSA
- NaSCN (Sodium thiocyanate); Fulka #71938; M=81.07
  Make a stock-solution of 6 M NaSCN (e.g. 97.28 g NaSCN in 200 ml PBS) and make the required concentrations out of this stock. Commonly used range: 0M - 0,25M - 0,5M - 0,75M - 1M - 1,25M - 1,5M - 1,75M - 2M - 2,25M - 2,5M - 3M.
- $MgCl_2*6H_2O$: (magnesium chloride hexahydrate) Merck #1.05833.0250; M=203.30
- Diethanolamine: Merck #803116; M=105.14
- DEA-buffer: 500 ml MQ + 492 µl Diethanolamine pH 9.8 + 0.15% w/v $MgCl_2*6H_2O$ (e.g. 0.15 g)
- PNPP (Para Nitro Phenyl Phosphate Hexahydrate); Fluka #71768

Method

1. Coat ELISA-plates with the desired coating. Coating diluted in CB, 100 µl/well. Incubate o/n at 4 °C, covered with a plastic seal or a lid.
2. Prepare samples and standards in deepwell plates. Dilute samples in DB, make 100 µl/well at a concentration of 1 AU. Incubate o/n at 4 °C.
3. Remove coating from plates by inverting the plates with a vigorous wrist action.
4. Block the plates with 200 µl/well BB, incubate 1h at RT.
5. Wash with platewasher program 9.
6. Add 100 µl /well of sample at 1 AU. Add 100 µl /well DB for the standard.
7. Add 100 µl of standard and dilute it 2-fold over 11 wells. Leave the Blank blank. Incubate 1h at RT.
8. Wash with platewasher program 9.

9. Add 100 µl /well NaSCN in different concentrations to the wells with samples. For the wells with standard or blank just add DB. Incubate 15 min at RT.
10. Wash with platewasher program 9.
11. Add 100 µl/well conjugated antibody diluted in DB. Incubate 1h at RT.
12. Wash with platewasher program 9.
13. Add 100 µl/well PnPP (1 mg/ml) in DEA-buffer. Incubate 30 min at RT.
14. Read OD at 450 nm on the platereader.
15. Export data as csv-file.
16. Calculate results using ADAMSEL.

Schematic:

Action	Material	Supplier	Concentration/ Dilution	Dilute in	Incubate
Coating	AMA-1, MSP-1, DiCo1, 2 or 3	BPRC	1 µg/ml	CB	o/n 4 °C
	Sh-α-Rb IgG	Sigma #R-3631	1/4000		
	G-α-M IgG	Sigma #A-4656	1/1000		
	G-α-Ra IgG	Pierce #31220	1/2000		
	G-α-Hu IgG	Sigma #I-3382	1/4000		
	G-α-Hu IgM	Sigma #I-2386	5 µg/ml		
Blocking				BB	1h RT
Samples	Standard curve			DB	1h RT
	Samples		1 AU	DB	
Elution	NaSCN-range	Fluka #71938	0 M – 3M	PBS	15 min RT
Conjugate	G-α-Rb IgG-AP	Pierce #31340	1/1250	DB	1h RT
	G-α-Ra IgG-AP	Pierce #31220	1/1250		
	G-α-M IgG-AP				
	G-α-Hu IgG-AP	Pierce #31310	1/1250		
	G-α-Hu IgM-AP	Sigma #A-2189	1/10000		
Substrate	PnPP	Fluka 71768	1 mg/ml	DEA buffer	30 min RT

Plateformat:

	1	2	3	4	5	6	7	8	9	10	11	12
A	St 1	St 1/2	St 1/4	St 1/8	St 1/16	St 1/32	St 1/64	St 1/128	St 1/256	St 1/512	St 1/1024	Blank
B	St 1	St 1/2	St 1/4	St 1/8	St 1/16	St 1/32	St 1/64	St 1/128	St 1/256	St 1/512	St 1/1024	Blank
C	S1 0M	S1 0.25M	S1 0.5M	S1 0.75M	S1 1M	S1 1.25M	S1 1.5M	S1 1.75M	S1 2M	S1 2.25M	S1 2.5M	S1 3M
D	S1 0M	S1 0.25M	S1 0.5M	S1 0.75M	S1 1M	S1 1.25M	S1 1.5M	S1 1.75M	S1 2M	S1 2.25M	S1 2.5M	S1 3M
E	S2 0M	S2 0.25M	S2 0.5M	S2 0.75M	S2 1M	S2 1.25M	S2 1.5M	S2 1.75M	S2 2M	S2 2.25M	S2 2.5M	S2 3M
F	S2 0M	S2 0.25M	S2 0.5M	S2 0.75M	S2 1M	S2 1.25M	S2 1.5M	S2 1.75M	S2 2M	S2 2.25M	S2 2.5M	S2 3M
G	S3 0M	S3 0.25M	S3 0.5M	S3 0.75M	S3 1M	S3 1.25M	S3 1.5M	S3 1.75M	S3 2M	S3 2.25M	S3 2.5M	S3 3M
H	S3 0M	S3 0.25M	S3 0.5M	S3 0.75M	S3 1M	S3 1.25M	S3 1.5M	S3 1.75M	S3 2M	S3 2.25M	S3 2.5M	S3 3M

Results

Calculate results using ADAMSEL.

In the sheet "Samples All" you find all the OD-values and calculated concentrations of the samples. To a new sheet you copy the following columns: "Name sample", "Conc1" and "Conc2" (Conc1 and Conc2 are the duplicates). Per sample the upper concentration is 100% bound.

In this new sheet, in the next column (next to the columns you just copied), you make 2 new columns "%1" and "%2". In these new columns you calculate the percentage remaining bound antibodies as: (Conc x/Conc 100)*100. You do this for both duplicates.

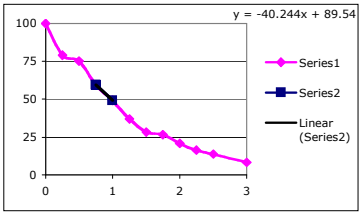
Then you make the next column "av%", where you take the average of the calculated percentages. And in the next column you put the [NaSCN].

Then you make a graph, on the x-axis the [NaSCN] and on the y-axis the percentage bound.

Within this graph you add the smaller line of just to 2 adjacent concentrations and percanteges around 50% (so in the example 59.4% at 0.75M and 49.3% at 1M), and from this line you add the trendline. Choose the option "Display equation on chart" as you add the trendline.

Next to the graph you copy this equation (in this example the equation is y = -40.244x + 89.54). To calculate 'x' you then put in 'y=50' (the formula will now become "(89.54-50)/40.244" resulting in "0.98"). In the formula 'x' is the the avidity-index.

Sample Name	Conc1	Conc2	%1	%2	gem%	[NaSCN]
102 BJ	1.20E-04	1.09E-04	100.0	100.0	**100.0**	**0**
102 BJ	9.09E-05	8.96E-05	76.0	82.0	**79.0**	**0.25**
102 BJ	8.68E-05	8.50E-05	72.6	77.7	**75.1**	**0.5**
102 BJ	6.76E-05	6.81E-05	56.5	62.2	**59.4**	**0.75**
102 BJ	6.25E-05	5.07E-05	52.3	46.3	**49.3**	**1**
102 BJ	4.21E-05	4.23E-05	35.2	38.6	**36.9**	**1.25**
102 BJ	3.03E-05	3.44E-05	25.3	31.5	**28.4**	**1.5**
102 BJ	3.01E-05	3.05E-05	25.2	27.9	**26.6**	**1.75**
102 BJ	2.21E-05	2.55E-05	18.5	23.3	**20.9**	**2**
102 BJ	1.92E-05	1.85E-05	16.0	16.9	**16.5**	**2.25**
102 BJ	1.67E-05	1.48E-05	14.0	13.6	**13.8**	**2.5**
102 BJ	9.02E-06	9.91E-06	7.5	9.1	**8.3**	**3**



	50% =	
102 BJ	0.98	y = -40.244x + 89.54