BAB V

KESIMPULAN dan SARAN

A. Kesimpulan

1. Pemberian Probiotik *Lactobacillus acidophilus* meningkatkan kadar IgA pada mencit yang diinduksi *Salmonella typhimurium.*
2. Pemberian Prebiotik ubi jalar meningkatkan kadar IgA pada mencit yang diinduksi *Salmonella typhimurium.*
3. Pemberian campuran Probiotik *Lactobacillus acidophilus* dan Prebiotik ubi jalar meningkatkan kadar IgA pada mencit yang diinduksi *Salmonella typhimurium.*
4. Terdapat perbedaan kadar IgA pada mencit yang diberi Probiotik *Lactobacillus acidophilus*, Prebiotik Ubi jalar dan keduanya.

B. Saran

Kepada peneliti selanjutnya diharapkan dapat mengembangkan variabel yang lebih baik dengan paparan bakteri atau dosis yang berbeda, memeriksa kadar IgA pada spesimen yang lain, melakukan variasi, menambah sampel, serta melakukan pemeriksaan parameter imun yang lain seperti produksi IgM, IgG, IgA serum, Proliferasi limfosit atau produksi sitokin pada *peyer's patch.* serta memperhatikan teknis-teknis yang akan dilakukan sehingga akan meminimalisir kesalahan.

Lampiran

Lampiran 1. Hasil pemeriksaan IgA

KODE	HASIL IGA
K1	**101.63**
K1	**107.85**
K1	**105.78**
K1	**112.38**
K1	**109.93**
K2	**87.96**
K2	**99.55**
K2	**97.48**
K2	**85.78**
K2	**101.63**
P1	**118.23**
P1	**118.23**
P1	**124.46**
P1	**128.61**
P1	**139.00**
P2	**114.08**
P2	**116.16**
P2	**120.31**
P2	**118.23**
P2	**114.08**
P3	**122.38**
P3	**132.77**
P3	**130.69**
P3	**136.92**
P3	**141.08**

Lampiran 2. Hasil SPSS

Descriptives

HASIL IgA

	N	Mean	Std. Deviation	Std. Error
KONTROL NEGATIF	5	107.5140	4.10049	1.83380
KONTROL POSITIF	5	94.4800	7.14192	3.19396
PROBIOTIK	5	125.7060	8.63854	3.86327
PREBIOTIK	5	116.5720	2.70700	1.21061
PROBIOTIK DAN PREBIOTIK	5	132.7680	7.04551	3.15085
Total	25	115.4080	14.92062	2.98412

Test of Homogeneity of Variances

HASIL IgA

Levene Statistic	df1	df2	Sig.
1.710	4	20	.187

ANOVA

HASIL IgA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4545.349	4	1136.337	28.492	.000
Within Groups	797.650	20	39.882		
Total	5342.998	24			

Tests of Normality

KELOMPOK		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
HASIL IgA	KONTROL NEGATIF	.136	5	.200*	.987	5	.967
	KONTROL POSITIF	.263	5	.200*	.871	5	.271
	PROBIOTIK	.207	5	.200*	.891	5	.363
	PREBIOTIK	.221	5	.200*	.902	5	.422
	PROBIOTIK DAN PREBIOTIK	.184	5	.200*	.978	5	.921

*. This is a lower bound of the true significance.

Multiple Comparisons

Dependent Variable: HASIL IgA

Bonferroni

(I) KELOMPOK	(J) KELOMPOK	Mean Difference (I-J)	Std. Error	Sig.
KONTROL NEGATIF	KONTROL POSITIF	13.03400*	3.99412	.039
	PROBIOTIK	-18.19200*	3.99412	.002
	PREBIOTIK	-9.05800	3.99412	.346
	PROBIOTIK DAN PREBIOTIK	-25.25400*	3.99412	.000
KONTROL POSITIF	KONTROL NEGATIF	-13.03400*	3.99412	.039
	PROBIOTIK	-31.22600*	3.99412	.000
	PREBIOTIK	-22.09200*	3.99412	.000
	PROBIOTIK DAN PREBIOTIK	-38.28800*	3.99412	.000
PROBIOTIK	KONTROL NEGATIF	18.19200*	3.99412	.002
	KONTROL POSITIF	31.22600*	3.99412	.000
	PREBIOTIK	9.13400	3.99412	.332
	PROBIOTIK DAN PREBIOTIK	-7.06200	3.99412	.923
PREBIOTIK	KONTROL NEGATIF	9.05800	3.99412	.346
	KONTROL POSITIF	22.09200*	3.99412	.000
	PROBIOTIK	-9.13400	3.99412	.332
	PROBIOTIK DAN PREBIOTIK	-16.19600*	3.99412	.006
PROBIOTIK DAN PREBIOTIK	KONTROL NEGATIF	25.25400*	3.99412	.000
	KONTROL POSITIF	38.28800*	3.99412	.000
	PROBIOTIK	7.06200	3.99412	.923
	PREBIOTIK	16.19600*	3.99412	.006

*. The mean difference is significant at the 0.05 level.

Lampiran 3. Perhitungan Dosis

A. Berat rata-rata mencit = 25 g

B. Bahan yang akan di sonde ke mencit adalah tablet Probiotik Lactobacillus acidophilus. Dosis tablet probiotik Lactobacillus acidophilus orang dewasa 1 gram. Dosis yang digunakan dalam penelitian adalah 1 gram.

C. Dosis konversi tablet probiotik Lactobacillua acidophilus dari 70 kgBB manusia untuk mencit :

   a. 0,0026 x 1000 mg = 2,6 mg/kgBB mencit

   b. Dosis untuk 1 ekor mencit = 2,6 x 1000/25 = 104 mg

   c. 104 mg diencerkan dengan aquadest steril 0,5 ml

Lampiran 4. KIT ELISA

Product Manual
Revision date: 17/May/2019

Mouse Immunoglobulin A (IgA) ELISA Kit

Catalog No.: abx350941

Size: 96T

Range: 6.25 ng/ml - 400 ng/ml

Sensitivity: 3.75 ng/ml

Storage: Store the 96-well plate, Standards, HRP-conjugate reagent and Biotin-conjugated antibody at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of IgA in Mouse Serum, Plasma and other biological fluids.

Introduction: Immunoglobulin A (IgA, also referred to as sIgA in its secretory form) is an antibody that plays a crucial role in the immune function of mucous membranes. The amount of IgA produced in association with mucosal membranes is greater than all other types of antibody combined. In absolute terms, between three and five grams are secreted into the intestinal lumen each day. This represents up to 15% of total immunoglobulins produced throughout the body.

Principle of the Assay
This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to IgA is pre-coated onto a 96-well plate. The standards and samples are added to the wells, incubated and washed with wash buffer. A biotin conjugated antibody specific to IgA is used for detection. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the IgA amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of IgA can be calculated.

Kit components
1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin conjugated antibody (Dilution 1:100): 120 µl
5. Antibody diluent buffer: 12 ml
6. HRP Conjugate Reagent (Dilution 1:100): 120 µl
7. HRP diluent buffer: 12 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml
11. Plate Sealer: 5

Material Required But Not Provided
1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 liter graduated cylinders

Product Manual
Revision date: 17/May/2019

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.
» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.
» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

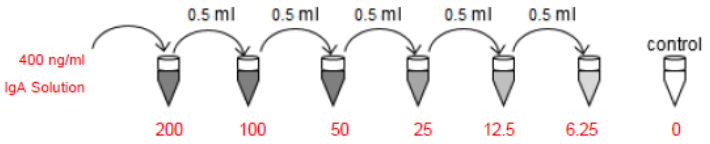
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Preparation of the IgA standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for 1 minute as the powder may drop off from the cap when opening. **(Note: Do not dilute the standard directly in the plate).** Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.

a.) 400 ng/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube. Allow the reconstituted standard to sit for 15 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles.

b.) 200 ng/ml → 6.25 ng/ml standard solutions: Label 6 tubes with 200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml and 6.25 ng/ml. Aliquot 0.5 ml of the Sample / Standard diluent buffer into each tube. Add 0.5 ml of the above 400 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.5 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.5 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard. Please use the diluted Standards for a single assay procedure and discard after use.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of antibody diluent buffer. Discard after use.

5. Preparation of HRP Conjugated Reagent working solution: prepare no more than 30 min. before the experiment
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the HRP Conjugate Reagent with HRP diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of HRP Conjugate Reagent into 99 µl of HRP diluent buffer. Discard after use.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. Add the solution at the bottom of each well without touching the side walls.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the liquid. Do not wash.
7. Add 100 µl of prepared Biotin conjugated antibody working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
8. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash Buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
9. Add 100 µl of HRP working solution into each well, cover the plate and incubate at 37°C for 30 minutes.
10. Remove the cover, discard the liquid and wash the plate 5 times with Wash Buffer as explained in step 8.
11. Add 90 µl of TMB substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference only, the optimal time should be determined by end user. Do not exceed 30 min.
12. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Mouse IgA concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of IgA were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of IgA were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

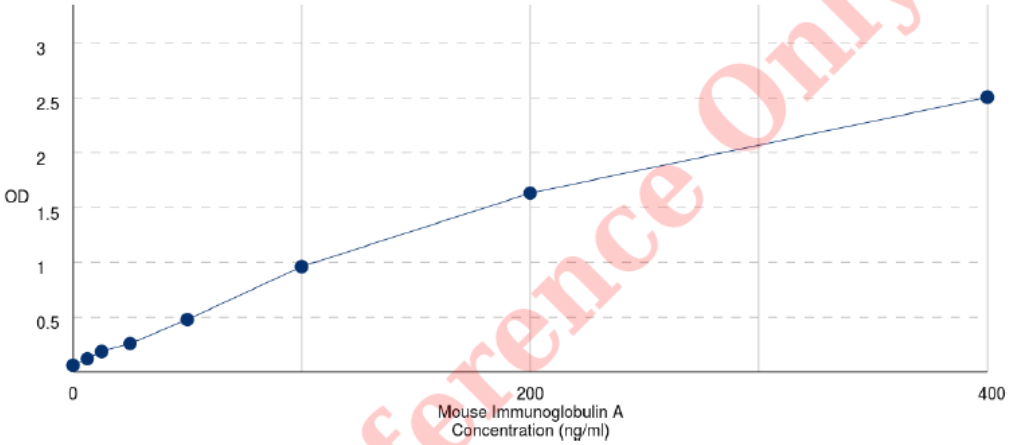
Intra-Assay: CV<10%

Inter-Assay: CV<10%

E. Typical Data & Standard Curve

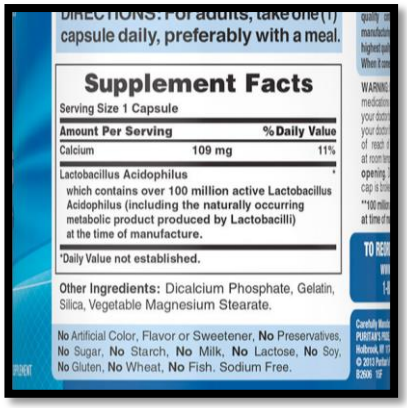
Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Concentration ng/ml	0	6.25	12.5	25	50	100	200	400
OD450	0.061	0.122	0.188	0.260	0.479	0.961	1.632	2.507



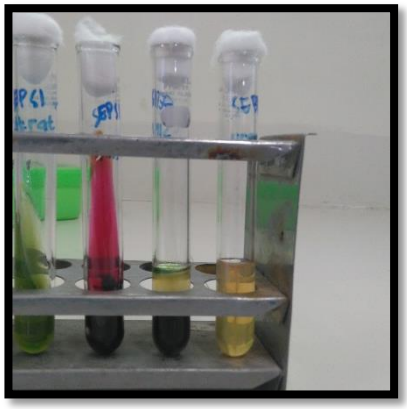
Lampiran 5. Mencit Balb/c

Lampiran 6. *L. acidophilus*



Lampiran 7. Komposisi Tablet *L. acidophilus*

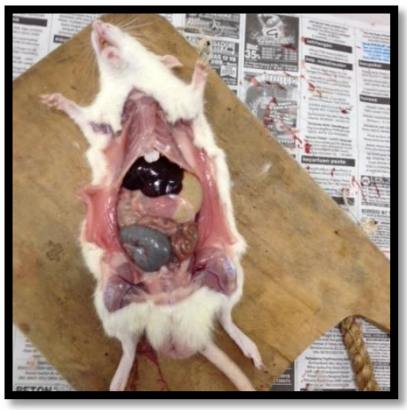
Lampiran 8. Ekstrak Ubi Jalar



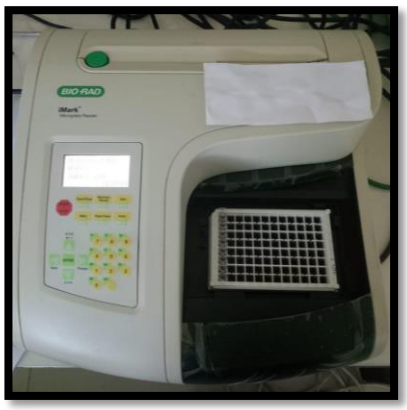
Lampiran 9. Media Uji Biokimia *Salmonella typhimurium*

Lampiran 10. Media SSA *Salmonella typhimurium*

Lampiran 11. Timbangan



Lampiran 12. Laparotomi Mencit Balb/c



Lampiran 13. Elisa Reader

Lampiran 14. Reagen ELISA

Lampiran 15. ELISA *well*

Lampiran 16. Sampel

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