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Catalogue No: TP 802M

PHASE™ RANGE

Mouse SAA ELISA kit

96 test kit

Instructions for use

For *in vitro* research use only.

Tridelta Development Ltd.

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Other “PHASE” acute phase assays available from Tridelta:

TP-801	Haptoglobin	Multispecies specific, colormetric, rapid assay may be used in a manual or on a large range of autoanalysers.
TP-801-Cal	Haptoglobin	Haptoglobin *calibrator* (2.5mg/ml) for Haptoglobin kit (TP-801)
TP-801-Con	Haptoglobin	Haptoglobin *controls* for Haptoglobin kit (TP-801)
TP-803	CRP-Canine	EIA C-reactive Protein assay specific for canine.
TP-803-Con	CRP-Canine	Canine CRP *controls* for Canine CRP EIA kits (TP-803).
TA-901	CRP-Porcine	EIA C-reactive Protein assay specific for porcine.
TP-802	SAA	Multispecies Serum Amyloid A immunoassay.
TP-802-Con	SAA	Multispecies SAA *control* for SAA kit (TP-802)
TP-802H	Human SAA	EIA Serum Amyloid A assay specific for human .
TP-807	Milk MAA	EIA Amyloid A specific for milk.
TP-802M-Con	SAA- Murine	Mouse SAA *controls* for SAA kit (TP-802M)

Clinical Study

A study was set up to investigate the acute phase response in three different strains of mice. Six mice of each of the following strains BALB/c, NIH inbred and ICR outbred were injected intraperitoneally with lipopolysaccharide (LPS). Two mice of each strain were given a medium dose of LPS (50ug), an additional two mice of each strain were given a high dose of LPS (500ug) and the final two mice of each strain were used as 'experimental' controls and were not given any LPS. The concentrations of two acute phase proteins SAA and Haptoglobin were monitored over an 18 hour period.

Table 6: Summary of results:

Mouse Strain	LPS Conc. adminstered	SAA (ug/ml)	Haptoglobin (mg/ml)
NIH	control	11.2	0.112
NIH	control	11.4	0.100
NIH	50ug	6076	1.364
ICR	control	8.0	0.094
ICR	control	11.8	0.115
ICR	50ug	3593	1.434
ICR	50ug	3244	1.511
ICR	500ug	2238	1.414
ICR	500ug	3336	1.437
Balb/c	control	11.8	0.162
Balb/c	50	3678	0.762
Balb/c	500	2552	0.360
Balb/c	500	3328	0.706

Limitations of the Procedure

The influences of various drugs, aberrant sera (haemolysed, hyperlipidemic, jaundiced, etc.) have not been investigated.

Intended Use

The mouse Serum Amyloid A ELISA is an in vitro assay for the quantitative determination of Serum Amyloid A (SAA) in mouse serum or plasma.

This kit has been developed for *in-vitro* research use only.

Background

Serum Amyloid A (SAA) is a low molecular weight acute phase protein produced in the liver in response to a series of stimuli such as viral or bacterial infection, trauma or other causes of inflammation and tissue damage. During an acute phase response the concentration of SAA in blood rises dramatically up to 1000-fold excess above normal circulating levels. SAA has been demonstrated to have utility in predicting renal allograft rejections and in assessing the inflammatory status of arthritis. SAA has also been used to monitor recovery from infections and inflammation, with the levels falling rapidly in response to therapy.

Assay Principle

The Tridelta Mouse SAA test is a solid phase ELISA. Mouse samples, including calibrators of known SAA content, are incubated in micro-wells at 37^{o}C together with a HRP labelled anti-SAA antibody. Any SAA present will be captured between the coated microplate and the labelled antibody. The plate is washed manually after sample and antibody-HRP incubation to remove any unbound material. Following the addition of TMB, a blue product is generated in direct proportion to the amount of SAA present in the original sample or calibrator. The reaction is stopped with the addition of stop reagent.

Reagents Provided:

1. SAA antibody coated wells 1 x 96 well plate
2. Wash buffer concentrate (red) 1 x 50ml **(20x concentrate)**
3. Sample/calibrator diluent concentrate (blue) 1 x 30 ml **(10x concentrate)**
4. SAA Calibrator 1 x freeze dried vial
5. Anti-SAA-HRP conjugate(Yellow) 1 x 6 ml **(Ready to Use)**
6. TMB 1 x 11 ml **(Ready to Use)**
7. Stop reagent 1 x 11 ml **(Ready to Use)**

Additional Materials Required:

1. Serum or plasma collection equipment.
2. Deionised or distilled water.
3. Plate washer: Automated or manual.
4. Glass or plastic dilution tubes.
5. Absorbent paper towels.
6. 37°C incubator.
7. Microtitre plate reader capable of measurement at 450nm with a reference at 630nm if available.
8. Accurate micropipettes and disposable tips to deliver 0-10ul, 20-200ul and 200-1000µl.
9. A repeat or multi-channel pipette (50-200µl) for large assays.
10. Plate Sealers to cover plate during assay.
11. Graph paper: Linear (Cartesian).
12. Timer.
13. Vortex.

C) Inter batch reproducibility data

Three mouse serum samples containing low, medium and high levels of SAA were assayed in replicates(16 times) in three consecutive production batches to determine **inter – batch** precision/reproducibility.

Table 4: Inter batch assay reproducibility

	Sample 1 (µg/ml)	**Sample 2 (µg/ml)**	**Sample 3 (µg/ml)**
n	48	48	48
Mean (ug/ml)	57.1	201.4	478
Standard Dev	3.7	9.2	29.6
%CV	6.0	4.5	6.2

Recovery

The recovery of known quantities of SAA spiked into both serum and plasma matrices, at three different concentrations, were evaluated.

Table 5: Recovery of SAA in serum and plasma

Matrix	**SAA (ug/ml)**	**Spiked SAA dilution**	**SAA recovered**	**Recovery %**
Serum	392	1:200	384	98
		1:400	412	105
		1:800	376	96
Plasma	117.6	1:200	111	95
		1:400	112	95
		1:800	111	95

Serum or plasma samples are recommended for use in this test. However, to eliminate potential discrepancies, it is also recommended that any study that starts with a particular matrix, serum or plasma, should continue to use the same matrix for the duration of the investigation.

Analytical sensitivity:

The analytical sensitivity of Ms SAA is 0.03 ug/ml (6.2 ug/ml when sample dilution of 1:200 is taken into account) determined by the addition of two standard deviations of the mean OD obtained when the zero calibrator was assayed 32 times.

Reproducibility:

A) Intra assay reproducibility data

Three mouse serum samples containing low, medium and high levels of SAA were assayed in replicates (16 times) to determine intra (within) assay precision/reproducibility.

Table 2: Intra (within) assay reproducibility

	Sample 1 (µg/ml)	**Sample 2 (µg/ml)**	**Sample 3 (µg/ml)**
n	16	16	16
Mean (ug/ml)	58.7	208.2	497.6
Standard Deviation	2.9	7.9	22.1
%CV	4.9	3.8	4.4

B) Inter assay reproducibility data

Three mouse serum samples containing low, medium and high levels of SAA were assayed in replicates(16 times) in three kits from the same production batch to determine **inter – assay** precision/reproducibility.

Table 3: Inter assay reproducibility

	Sample 1 (µg/ml)	**Sample 2 (µg/ml)**	**Sample 3 (µg/ml)**
n	48	48	48
Mean (ug/ml)	56.6	224.3	560.4
Standard Dev	4.1	15.7	54.7
%CV	7.2	7.0	9.7

Precautions

Safety

- For *in vitro* research purposes only.
- Some reagents contain thimerosal and may be toxic if ingested.
- Dispose of all clinical specimens, infected or potentially infectious material in accordance to good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
- Never pipette by mouth and never eat or drink at the laboratory workbench.
- Wear disposable latex gloves and eye protection where appropriate.
- The stop solution and TMB contain reagents that may irritate the skin or mucous membranes. Any reagent, which comes into contact with the skin, should be washed off with water immediately.
- Wash hands thoroughly when finished.

Procedural

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from protocol provided may cause erroneous results.
- Samples should be stored refrigerated, or frozen if they are not to be analysed shortly after collection. Avoid repeated freeze thaw cycles.
- When possible avoid the use of badly haemolysed or lipemic sera. If large amounts of particulate matter are present this should be removed by centrifugation prior to assay.
- It is recommended that all calibrators and samples are ran in duplicate.
- Allow all reagents to come to room temperature (20 – 25^{0}C) and mix well before use.
- Avoid leaving reagents in direct sunlight and/or above 4^{0}C for extended periods.
- Cover or cap all reagents when not in use.
- High quality distilled or deionised water is required for the Wash Solution and Diluent Buffer. The use of contaminated water may lead to background colour in the assay.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Care must be taken not to contaminate components and always use fresh tips for each sample and component.

- Reagent delivery should be aimed at the midpoint of the side of microtitration wells, taking care not to scratch the side with the pipette tip.
- Do not allow microwells to dry at any stage during the procedure. Never insert absorbent paper directly into the wells.
- Ensure that the bottom surface of the well is clean and dry before reading.
- Before commencing the assay an identification and distribution plan should be established. It also recommended labelling each strip to enable identification.
- Read absorbances shortly after completion of the assay.
- SAA values should only be determined from the 'linear portion' of the curve.

Directions for Washing

Incomplete washing will adversely affect the test outcome. All washing must be performed with the wash buffer (red), 1x, provided with the kit.

Washing is performed **manually** as follows: Completely aspirate the liquid from all wells by inverting the plate or gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 400µl of diluted wash solution and then aspirate as outlined above. Repeat as directed under 'Assay Procedure'. After the washing procedure, the plate is inverted and tapped dry on adsorbent tissue.

Storage

The kit components are stable when stored at 2-8^{o}C until the expiry date indicated on the kit label. Note: The stability of reagents which require dilution will have a reduced shelf life once prepared. Refer to the specific section of each reagent in "Reagent Preparation".

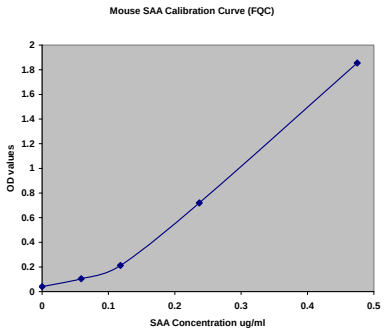
Interpretation of Test Results

1. Calculate the mean absorbance for each sample, control or calibrator.
2. Plot the **absorbance** of the calibrators against the SAA **concentration** on cartesian graph paper. (If necessary, the background absorbance for the 0ng/ml calibrator may be subtracted from each of the data points, including the calibrators, unknowns and controls prior to plotting). Draw the best smooth curve through these points to construct the calibration curve.
3. Determine the concentrations of the test samples and controls from the calibration curve by multiplying the interpolated value by the appropriate dilution factor. Samples that have a signal greater than the highest standard should be further diluted in diluent buffer and re-analysed to fall on the linear part of the curve.

Performance Characteristics

Typical Data

An example of a typical calibration curve is represented below. This should not be used in the determination of SAA concentration.



Summary of Assay Procedure

Summary Ms SAA
Coated microplate: 96 well
50 µl (ready to use) Anti-SAA HRP conjugate (yellow) + **50 µl** calibrator, control or mouse sample(1:200)
Incubate 1 hour at 37^0C
Wash *(manually)* 4 times
100 µl TMB substrate solution
Incubate 15 min at room temperature (Do **NOT** wash)
100 µl stop solution
Read absorbance at 450 nm

Method

Reagent Preparation

Diluent buffer (Blue), 1x

Dilute 1 volume of diluent buffer concentrate (10x) with 9 volumes of distilled water. Prepared reagent is stable for one day at room temperature and should not be stored for extended periods.

NOTE: Ensure that any crystals that may have developed in the diluent buffer have been completely dissolved prior to dilution.

Wash buffer (Red),1x

Dilute 1 volume of wash buffer concentrate (20x) with 19 volumes of distilled water. Store both the wash buffer concentrate and working wash buffer (1x) in the refrigerator. Diluted wash solution is stable for up to 2 weeks when stored at 4^0C.

Dilution of SAA calibrators

Note: The calibration curve should be prepared after all samples and controls have been diluted and immediately before plate loading.

1. To prepare the top calibrator, reconstitute the SAA calibrator provided in the kit by adding 1ml of diluent buffer,1x, to the vial. Vortex vigorously to dissolve completely.
2. Label 5 tubes C1-C5. Add 300ul of the top calibrator to the first tube labelled C1. Immediately aliquot (320ul per aliquot) and freeze remaining unused top calibrator material at -20^0C.
3. Add 150ul of diluent buffer, 1x, to the remaining 4 tubes labelled C2-C5 respectively, where tube C4 represents the lowest calibrator with SAA and C5 is the zero calibrator or assay blank. (Diluent buffer only).
4. Add 150ul of the top calibrator (C1) to tube C2. Mix well and serially dilute down to complete the range as directed in Table 1.
5. Discard all diluted calibrators immediately after use and prepare a new range as required from the frozen stock calibrator – step 2 above.

Table 1: Preparation of Working Calibration Curve

Tube Number	Concentration ug/ml	Volume of kit calibrator (ul)	Volume of Diluent buffer(ul)	Serial Dilution
C1	0.500	300	--	---
C2	0.250	-	150	150 of C1
C3	0.125	-	150	150 of C2
C4	0.062	-	150	150 of C3
C5	0.000	-	150	-

-

Note: The 'working assay range' is 12.5 - 100 µg/ml when the sample dilution of 1:200 is taken into account.

Specimen Preparation

Serum /Plasma

Specimens should be collected by venipuncture into serum or plasma collection tubes. Blood samples may be kept for up to 24 hours before separation of plasma or serum. However, it is best to remove serum from the clot or cells and debris from plasma as soon as possible after collection. In general, serum or plasma may be stored at 2-8 ^{o}C for up to 24 hours before screening or alternatively stored longterm frozen at -20 ^{o}C for up to one year. Repeated freeze thaw cycles do not appear to affect the SAA concentration.
It is important that all samples are brought to room temperature and vortexed vigorously to assure accurate determination of the SAA concentration.
Do not use grossly haemolysed or lipaemic samples.

All samples require a final dilution of 1:200 prior to testing. Normal serum or plasma levels of SAA are generally <20 µg/ml. Serum or plasma should be diluted by addition of 10ul of sample to 2 ml of working diluent buffer (1x). It is essential that samples are well vortexed before analysing.

Assay Procedure

Allow test reagents and samples to reach room temperature before use.

Note: ***Follow washing instructions rigorously as directed above under 'Directions For Washing'.*** A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Re-bag extra strips, seal bag and store in a refrigerator.
2. Vortex the serum or plasma samples to be examined by the test. Dilute samples 1:200, in sample diluent buffer, as recommended. Following sample dilution prepare the calibration curve as described in the table 1 above.
3. Add **50µl** of Anti-SAA/HRP (yellow) conjugate to each well.
4. Add **50µl**, in duplicate, of diluted calibrator, control or sample to each well. Tap sides of the plate gently to mix.
5. Cover the plate with a dust cover. Incubate the plate for **1 hour at 37^{o}C**.
6. After incubation aspirate or decant well contents. Wash the whole plate manually as directed under 'Directions for Washing' Repeat **4 times** ensuring that wells do not remain empty during wash cycles. After the last wash, tap the plate dry on absorbent paper.
7. Add **100µl** of TMB substrate
8. Cover the plate and incubate at **room temperature for 15 minutes**.
9. Add **100µl** of stop solution and tap gently to mix.
10. Read the absorbance of each well at 450nm using 630nm as a reference, if available.