Reagent Preparation Sheet.

Preparation of Biotinylated MAB211p, SeM detector antibody in ELISA protocol.

Date prepared:

By:

I. **Hazard Assessment**

To protect yourself from any possible hazards associated with this task wear eye protection. You should also wear latex, nitrile, or vinyl gloves and a lab coat with long sleeves. To protect your legs and feet wear closed shoes and long trowsers. Do not wear sandles, shorts or a short skirt. Wash your hands before eating and when leaving the laboratory. You should review the MSDS for any chemical used in this procedure. In case of a spill with a toxic chemical remove all contaminated clothing and wash affected areas with copious quantities of water. Check location of the nearest safety shower. Eyes should be washed copiously for 15 minutes.

II. **Reagent**
Biotin conjugated MAB211p.

III. **Purpose of reagent**
The monoclonal IgG antibody used in detection of SeM antigen of *Streptococcus equi* subspecies *equi*. In ELISA format , it is used in conjunction with MAB212, the latter being a capture antibody to concentrate target on the ELISA plate.

IV. **Reconstitution and storage of Biotin**

Dissolve vial of biotin (10mg) in 250 ul of DMSO. This gives a final concentration of 40 mg/mL ____

Make 50 ul aliquots of biotin at 40 mg/mL (i.e. 5 aliquots at 50 ul) and store at - 20 C ____

V. **Biotinylation procedure**

In a 1.5 mL microfuge tube combine 12 (50 ul) vials of MAB211p (tracer antibody) (12 X 75ug = 900ug of MAB211p and 12 X 50ul = 600 ul total volume) ____

To this add 5 ul of biotin (5 ul X 40 mg/mL = 0.2 mg) ____

Mix and incubate at room temperature for an hour ____

Dialyse this volume (600 uL) against 0.15M PBS using a 2 mL Slide-A-Lyzer MINI Dialysis Device for 2 hours at room temperature, twice with 20 kD Molecular weight cut-off (20K MWCO). ____

Reconstitute with PBS up to a volume of 900 uL (~1 mg/mL) and make 50 ul aliquots (each at ~1 mg/mL i.e. 50 ug) and freeze aliquots at -20 C. ____

When ready to conduct ELISA, add 1 aliquot of biotinylated antibody (50 ug) to 10 mL of blocking buffer (0.15 M PBS with 1 % NFDM) to have a final concentration of 0.5 ug/mL of detector antibody in blocking buffer. ____

VI. Assessment of biotinylation using HABA/AVIDIN BIOTIN replacement assay

The assay provides a colometric assessment of displacement of HABA from its complex with AVIDIN by biotin. The HABA/AVIDIN complex is measured at A500, and its reduction in absorbance can be directly correlated to moles of HABA being replaced by biotin.

Reconstitute HABA/AVIDIN vial (Sigma H 2153) with 10 mL of deionized water. (Use needle and syringe.)	____	
Label vial "exp" and date one month from today, store at 0-4 C	____	
Add 180 ul of HABA/AVIDIN solution to well. Shake 5 min	____	
Record absorbance at 500 nm	____	A500 HA
Record concentration of biotinylated MAB211p mg/mL	____	mg/ml mgmlpro
Add 20 ul of biotinylated MAB 211p to same well. Shake 5 min	____	
Record absorbance at 500 nm	____	A500HAB
(If absorbance < 0.15 dilute sample and repeat assay)		
Calculate molar ratio of biotin to protein (target is 5 - 8)	____	**moles of biotin per mole of protein**

VII. R code Calculations

In the formula below fill in values xxx for mgmlpro, A500HA, and A500HAB. The formula generates the biotin to protein molar ratio and an array with values including DA (difference in absorbance), mmol per mL of biotin (mmolmlbio), mmol per mL of protein (mmolmlpro) and the last value is also the biotin to protein molar ration (bio2pro).

Formula for microplate

```
mgmlpro=xxx; A500HA=xxx; A500HAB=xxx;DA=(A500HA)-A500HAB;
mmolmlbio=DA/(34000*0.5);mmolmlpro=mgmlpro/150000;bio2pro=
(mmolmlbio*10)/mmolmlpro;bio2pro; rbind(mgmlpro,A500HA, A500HAB, DA,
mmolmlpro,mmolmlbio,bio2pro)#for microplate
```

In R paste the text after the command prompt ">". For example:

```
> mgmlpro=0.69; A500HA=0.904; A500HAB=0.771;DA=(A500HA)-A500HAB;
mmolmlbio=DA/(34000*0.5);mmolmlpro=mgmlpro/150000;bio2pro=
(mmolmlbio*10)/mmolmlpro;bio2pro; rbind(mgmlpro,A500HA, A500HAB, DA,
mmolmlpro,mmolmlbio,bio2pro)#for microplate

[1] 17.00767
              [,1]
mgmlpro   6.900000e-01
A500HA    9.040000e-01
A500HAB   7.710000e-01
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DA	1.330000e-01
mmolmlpro	4.600000e-06
mmolmlbio	7.823529e-06
bio2pro	1.700767e+01

Formula for cuvette.

mgmlpro=xxx; A500HA=xxx; A500HAB=xxx;DA=(0.9*A500HA)-A500HAB; mmolmlbio=DA/(34000*1);mmolmlpro=mgmlpro/150000;bio2pro= (mmolmlbio*10)/mmolmlpro;bio2pro; rbind(mgmlpro,A500HA, A500HAB, DA, mmolmlpro,mmolmlbio,bio2pro)#for cuvette

Refer to instructions for HABA/AVIDIN for how to perform assay in a cuvette system. The formula is adjusted slightly to adjust for change in absorbance with addition of sample (0.9), and different pathwidth of cuvette (1 cm) versus microtitre plate (0.5 cm)

VIII. Comments

Biotinamidocaproate N-Hydroxy-Succinimide ester provides more detailed information about biotin

(Sigma H 2153) gives detailed information about the HABA/AVIDIN reagent from Sigma used in this protocol, including instructions on how to reconstitute and store reagent.

HABA/AVIDIN gives detailed explanation and background information on the assay, and how to perform calculations. But instructions on how to reconstitute the vial are not applicable to this protocol since we use a different product (Sigma H 2153)

Slide-A-Lyzer detailed explanation and background information for slide-alyzer dilaysis system

Ordering Information:

www.Sigmaaldrich.com

Biotin - Biotinamidohexanoic acid N-hydroxysuccinimide ester 10 mg # B2643-10MG $29.90

Dimethyl sulfoxide CHROMASOLV Plu # 34869-100ML $66.30

HABA/Avidin Reagent lyophilized powder # H2153-1VL $39.50

www.piercenet.com

Slide-A-Lyzer MINI Dialysis Device, 20 Kdalton Molecular Weight Cut-Off (20K MWCO), 2mL (pack of 25 devices) catalog # 88405 $220.