

## **10. APPENDIX**

### **10.1 Reagents**

#### **10.1.1 Reagents for antigen preparation**

##### **Washing solution**

- Hank's solution 40.0 ml
- Penicillin/streptomycin (10.000 IU/ml) 20.0 ml
- Distilled H<sub>2</sub>O 360.0 ml

##### **Culture medium**

- Dulbecco modified Eagle's medium 100 ml
- Penicillin/streptomycin (10.000 IU/ml) 0.5 ml
- HEPES buffer solution 1.0 ml
- L-Glutamin 1.0 ml

#### **10.1.2 Reagent for ELISA test**

##### **Carbonate buffer pH 9.6**

- Na<sub>2</sub>CO<sub>3</sub> 1.12g
- NaHCO<sub>3</sub> 2.92 g
- NaN<sub>3</sub> 0.20 g
- dist. H<sub>2</sub>O ad 1000 ml

##### **Phosphate buffered saline/Twen20 (PBS-T) pH 7.3-7.4**

- NaCl 16.0 g
- KCl 0.4 g
- Na<sub>2</sub>HPO<sub>4</sub> x 12 H<sub>2</sub>O 5.8 g
- KH<sub>2</sub>PO<sub>4</sub> 0.4 g
- Tween 20 1.0 ml
- dist. H<sub>2</sub>O ad 2000 ml.

##### **ABTS solution**

ABTS (Sigma): 2, 2'-azino-bis (3-ethylbenzthiazoline 6 sulfonic acid)

- 2 tablets solved in 20.0 ml dist. H<sub>2</sub>O.

### 10.1.3 Reagents for SDS-PAGE

#### Sample buffer pH: 6.8

- Tris 6.06 g
- SDS 20% 2.00 ml
- $\text{NaN}_3$  0.01 g
- dist.  $\text{H}_2\text{O}$  ad 80 ml
- with HCl 4M adjust pH: 6.8, then full ad 100 ml with dist.  $\text{H}_2\text{O}$ .

#### Not reductive sample buffer

- SDS 20% 20 ml
- EDTA 0.003 g
- $\text{NaN}_3$  0.001 g
- Bromophenol blue 0.010 g
- Sample buffer 2.5 ml
- dist.  $\text{H}_2\text{O}$  ad 100 ml

#### DTT solution

- dithiothreitol 0.125 g
- dist.  $\text{H}_2\text{O}$  0.25 ml

#### Reductive sample buffer

- Not reductive sample buffer 10 ml
- DTT solution 0.1 ml

#### Coomassie solution

0.02% Coomassie Brilliant Blue R 350 + 10% acetic acid solution. Destaining in acetic acid 10%.

#### 10.1.4 Reagents for Immunoblot

##### Anode I buffer

- Tris 0.3 M 36.3 g
- Methanol 20% 200 ml
- dist. H<sub>2</sub>O ad 2000 ml.

##### Anode II buffer

- Tris 25mM 3.03
- Methanol 20% 200 ml
- dist. H<sub>2</sub>O ad 2000 ml.

##### Catode buffer

- 6-aminohexan acid 5.2 g
- SDS 0.01% 0.1 g
- Methanol 20% 200 ml
- dist. H<sub>2</sub>O ad 2000 ml.

##### Amido black staining

Solve 0.1 g Amido black in 100 ml methanol - acetic acid – water (40:10:50 v/v). Destaining in methanol – acetic acid – water 25:10:65 v/v).

##### Ponceau red staining

0.1% Ponceau Red in 0.1% acetic acid solution. Destaining in distilled H<sub>2</sub>O.

##### TBS buffer

- NaCl 1.5M 8.78 g
- Tris-Base 0.1 M 2.11 g
- NaN<sub>3</sub> 0.01 M 65 mg
- dist. H<sub>2</sub>O ad 2000 ml

##### Serum and coniugate buffer

TBS buffer with with 1 % Bovine Seroalbumine (BSA) and 0.05% Tween 20.

##### Blocking buffer

TBS buffer with 1 % BSA.

##### Washing buffer

TBS buffer with 0.05% Tween 20.

**Substrate solution**

BCIP/NBT solution, 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (SIGMA  
*Fast* BCIP/NBT tablets, one tablet in 10 ml dist. H<sub>2</sub>O).

## 10.2 TABLES

**Table 8. OD values of experimentally infected lambs sera against E/S antigen.**

E/S antigen						
day p.i.	A. 1000	B. 1000	C. 2000	D. 2000	control 1	control 2
0	0.129	0.108	0.124	0.078	0.169	0.102
15	0.139	0.177	0.161	0.190	0.293	0.192
30	0.616	0.356	0.433	0.482	0.291	0.233
45	0.848	0.574	1.157	0.743	0.295	0.226
60	1.017	-	1.327	1.442	0.268	0.191
90	1.122	-	1.337	1.536	0.312	0.293
120	0.932	-	1.103	0.976	0.299	0.169
150	1.216	-	1.365	0.978	0.268	0.306

**Table 9. OD values of experimentally infected lambs sera against somatic antigen.**

Somatic antigen						
day p.i.	A. 1000	B. 1000	C. 2000	D. 2000	control 1	control 2
0	0.177	0.163	0.232	0.172	0.286	0.246
15	0.193	0.283	0.218	0.257	0.235	0.287
30	0.531	0.630	0.411	0.441	0.363	0.267
45	0.811	0.616	1.156	0.722	0.378	0.328
60	0.855	-	1.278	1.255	0.340	0.367
90	1.002	-	1.195	1.257	0.324	0.363
120	0.894	-	1.154	1.133	0.377	0.340
150	1.313	-	1.439	1.184	0.342	0.314

**Table 10. OD values of sera from sheep naturally infected by *F.hepatica* against E/S and somatic *D.dendriticum* antigens.**

Sample	E/S Ag	So Ag
s1	0.175	0.245
s2	0.325	0.472
s3	0.247	0.343
s4	0.198	0.245
s5	0.688	0.710
s6	0.344	0.289
s7	0.409	0.395
s8	0.063	0.213
s9	0.165	0.096
s10	0.228	0.188
s11	0.323	0.421

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**Table 11. OD values of sera from experimentally infected sheep with *Nematodirus battus*, *Trichostrongylus colubriformis*, *Haemonchus contortus*, single infection dose**

Sample	E/S Ag	So Ag
1	0.123	0.156
2	0.122	0.161
3	0.097	0.086
4	0.213	0.118
5	0.112	0.135
6	0.085	0.314
7	0.113	0.138
8	0.125	0.101
9	0.259	0.142
10	0.255	0.142

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**Table 12. OD values of sera from experimentally infected sheep with *Dictyocaulus filaria*, *Oesophagostomum venulosum*, *Ostertagia ostertagi*, *T. colubriformis*, *Cooperia curticiei*, *N. battus*, four repeated infection dose.**

Sample	E/S Ag	So Ag
1	0.594	0.609
2	0.663	0.933
3	0.318	0.176
4	0.467	0.361
5	0.406	0.411
6	0.544	0.596
7	0.488	0.283