KITS USED IN EXPERIMENTS

I. ELISA kits

RayBio®
Mouse IL-2 ELISA Kit

User Manual
(Revised Mar 1, 2012)

RayBio® Mouse IL-2 ELISA Kit Protocol

(Cat#: ELM-IL2-001)

RayBiotech, Inc.

RayBiotech, Inc.

RayBio® Mouse IL-2 ELISA Kit Protocol

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RayBio®
Mouse IFN-γ ELISA Kit

User Manual
(Revised Mar 1, 2012)

RayBio® Mouse IFN-γ ELISA Kit Protocol

(Cat#: ELM-IFNgamma-001)

RayBiotech, Inc.

RayBiotech, Inc.

RayBio® Mouse IFN-γ ELISA Kit Protocol

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RayBio®
Mouse IL-4 ELISA Kit

User Manual
(Revised Mar 1, 2012)

RayBio® Mouse IL-4 ELISA Kit Protocol

(Cat#: ELM-IL4-001)

RayBiotech, Inc.

RayBiotech, Inc.

RayBio® Mouse IL-4 ELISA Kit Protocol

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RayBio®Mouse IL-10 ELISA Kit

User Manual
(Revised Mar 1, 2012)

RayBio® Mouse IL-10 ELISA Kit Protocol

(Cat#: ELM-IL10-001)

RayBiotech, Inc.

RayBiotech, Inc.

RayBio® Mouse IL-10 ELISA Kit Protocol

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II. Biochemical kits for studying liver and kidney functions

Coral Clinical Systems — Quick Reference Guide — **SGOT (ASAT) KIT**

SGOT (ASAT) Kit
Reitman & Frankel's Method

Intended Use:

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle and kidneys. Injury to these tissues results in the release of the enzyme in blood stream. Elevated levels are found in myocardial infarction, cardiac operations, hepatitis, cirrhosis, acute pancreatitis, acute renal diseases, primary muscle diseases. Decreased levels may be found in pregnancy, beri beri and diabetic ketoacidosis. SGOT (ASAT) kit uses the Reitman & Frankel's method to determine SGOT (ASAT) in serum.

SGOT (ASAT) Kit components:

L1	Substrate Reagent
L2	DNPH Reagent
L3	NaOH Reagent (4N)
S	Pyruvate Standard (2mM)
Other Accessories	Package Insert

Coral Clinical Systems — Quick Reference Guide — **SGPT (ALAT) KIT**

SGPT (ALAT) Kit
Reitman & Frankel's Method

Intended Use:

SGPT is found in a variety of tissues but it is mainly found in the liver. Increased levels are found in hepatitis, cirrhosis, obstructive jaundice and other hepatic diseases. Slight elevation of the enzymes is also seen in myocardial infarction. SGPT (ALAT) kit uses the Reitman & Frankel's method to determine SGPT (ALAT) in serum.

SGPT (ALAT) Kit components:

L1	Substrate Reagent
L2	DNPH Reagent
L3	NaOH Reagent (4N)
S	Pyruvate Standard (2mM)
Other Accessories	Package Insert

Coral Clinical Systems

Quick Reference Guide

LACTATE DEHYDROGENASE (P-L) KIT

Lactate Dehydrogenase (P-L) Kit
Mod. IFCC Method

Intended Use:

Lactate dehydrogenase (LDH) is found in many body tissues particularly heart, liver, skelatal muscle, kidney and RBCs. LDH is found in the form of isoenzymes based on their electrophoretic mobility with each isoenzyme being primarily from different organs. Increased levels are found in myocardial infarction, pulmonary diseases, hepatic diseases, hemolytic anemias, renal diseases and muscular dystrophy. LDH (P-L) kit uses the Mod. IFCC Method to determine lactate dehydrogenase in serum.

Lactate Dehydrogenase (P-L) Kit components:

L1	Buffer Reagent
L2	Starter Reagent
Other Accessories	Package Insert

Coral Clinical Systems

Quick Reference Guide

GAMMA GLUTAMYL TRANSFERASE KIT

Gamma Glutamyl Transferase Kit
Carboxy Substrate Method

Intended Use:

Gamma glutamyl transferase (GGT) is an enzyme found mainly in serum from hepatic origin, though the highest levels are in the kidneys. Elevated levels are found in hepatobiliary and pancreatic diseases. Chronic alcoholism, myocardial infarction with secondary liver damage and diabetics. Gamma glutamyl transferase kit uses the carboxy substrate method to determine gamma glutamyl transferase in serum.

Gamma Glutamyl Transferase Kit components:

L1	Buffer Reagent
T1	Substrate Tablets
Other Accessories	Package Insert

Coral Clinical Systems

Quick Reference Guide **ALKALINE PHOSPHATASE KIT**

Alkaline Phosphatase kit
Mod. Kind & King's Method

Intended Use:

Alkaline Phosphatase (ALP) is an enzyme of the hydrolase class of enzymes and acts in an alkaline medium. It is found in high concentrations in the liver, biliary tract epithelium and in bones. Normal levels are age dependent and increase during bone development. Increased levels are associated mainly with liver and bone diseases. Moderate increases are seen in Hodgkins diseases and congestive heart failure. Alkaline Phosphatase kit uses the Mod. Kind & King's method to determine alkaline phosphatase activity in serum.

Alkaline Phosphatase Kit components:

L1	Buffer Reagent
L2	Substrate Reagent
L3	Color Reagent
S	Phenol Standard (10 mg/dl)
Other Accessories	Package Insert

Coral Clinical Systems

Quick Reference Guide **ACID PHOSPHATASE KIT**

Acid Phosphatase Kit
Mod. King's Method

Intended Use:

Acid Phosphatase (ACP) is an enzyme of the hydrolase class of enzymes and acts in an acidic medium. It is widely distributed and found in high concentrations in the liver, RBC's and the prostrate. Increased levels of the prostatic fraction are associated with prostatic carcinomas. Increased levels of the non prostatic fraction are associated with liver disease, hyperparathyroidism and Paget's disease.

Acid Phosphatase kit is used for the determination of acid phosphatase activity in serum using the Mod. King's Method.

Acid Phosphatase Kit components:

L1	Buffer Reagent
L2	Substrate Reagent
L3	Colour Reagent
L4	Tartrate Reagent
S	Phenol Standard (10 mg/dl)
Other Accessories	Package Insert

Bilirubin Kit
Mod. Jendrassik & Grof's Method

Intended Use:

Bilirubin is mainly formed from the heme portion of aged or damaged RBCs. It then combines with albumin to form a complex which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver this bilirubin complex is combined with glucuronic acid into a water soluble conjugate. This is referred to as conjugated or direct bilirubin. Elevated levels of bilirubin are found in liver diseases (hepatitis and cirrhosis), excessive hemolysis / destruction of RBC (hemolytic jaundice) obstruction of the biliary tract (obstructive jaundice) in the drug induced reactions. The differentiation between the direct and indirect bilirubin is important in diagnosing the cause of hyperbilirubinemia. Bilirubin kit uses mod. Jendrassik & Grof's method to determine direct & total bilirubin in serum.

Bilirubin Kit components:

L1	Direct Bilirubin Reagent
L2	Direct Nitrite Reagent
L1	Total Bilirubin Reagent
L2	Total Nitrite Reagent
S	Artificial Standard (10 mg/dl)
Other Accessories	Package Insert

Urea Kit
Diacetyl Monoxime (DAM) Method

Intended Use:

Urea is the end product of protein metabolism. It is synthesized in the liver from the ammonia produced by the catabolism of amino acids. It is transported by blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shock, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy. Urea kit uses DAM method to determine urea in serum, plasma & urine.

Urea Kit components:

L1	Urea Reagent
L2	Acid Reagent
L3	DAM Reagent
S	Urea Standard (40 mg/dl)
Other Accessories	Package Insert

Creatinine Kit
Alkaline Picrate Method

Intended Use:

Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscle. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunction, reduced renal blood flow (shock, dehydration, congestive heart failure) diabetes acromegaly. Decreased levels are found in muscular dystrophy. Creatinine kit uses the alkaline picrate method to determine creatinine in serum and urine.

Creatinine Kit components:

L1	Picric acid Reagent
L2	Buffer Reagent
Standard	Creatinine Standard (2 mg/dl)
Other Accessories	Package Insert

System Parameters			
Reaction	: End Point	**Interval**	: ---
Wavelength	: 520 nm	**Sample Vol.**	: 0.20 ml
Zero Setting	: Reagent Blank	**Reagent Vol.**	: 1.10 ml
Incub. Temp	: R.T.	**Standard**	: 2 mg/dl
Incub. Time	: 20 min.	**Factor**	: ---
Delay Time	: ---	**React. Slope**	: Increasing
Read Time	: ---	**Linearity**	: 8 mg/dl
No. of read	: ---	**Units**	: mg/dl

Storage / Stability	Temperature	Duration
Unopened kit	15-30°C	24 Months
Opened kit (Unmixed)	15-30°C	24 Months
In use stability	2-8°C	1 Day

Available Pack Sizes	
15 Tests	35 Tests

Photographs of some of the experiments conducted

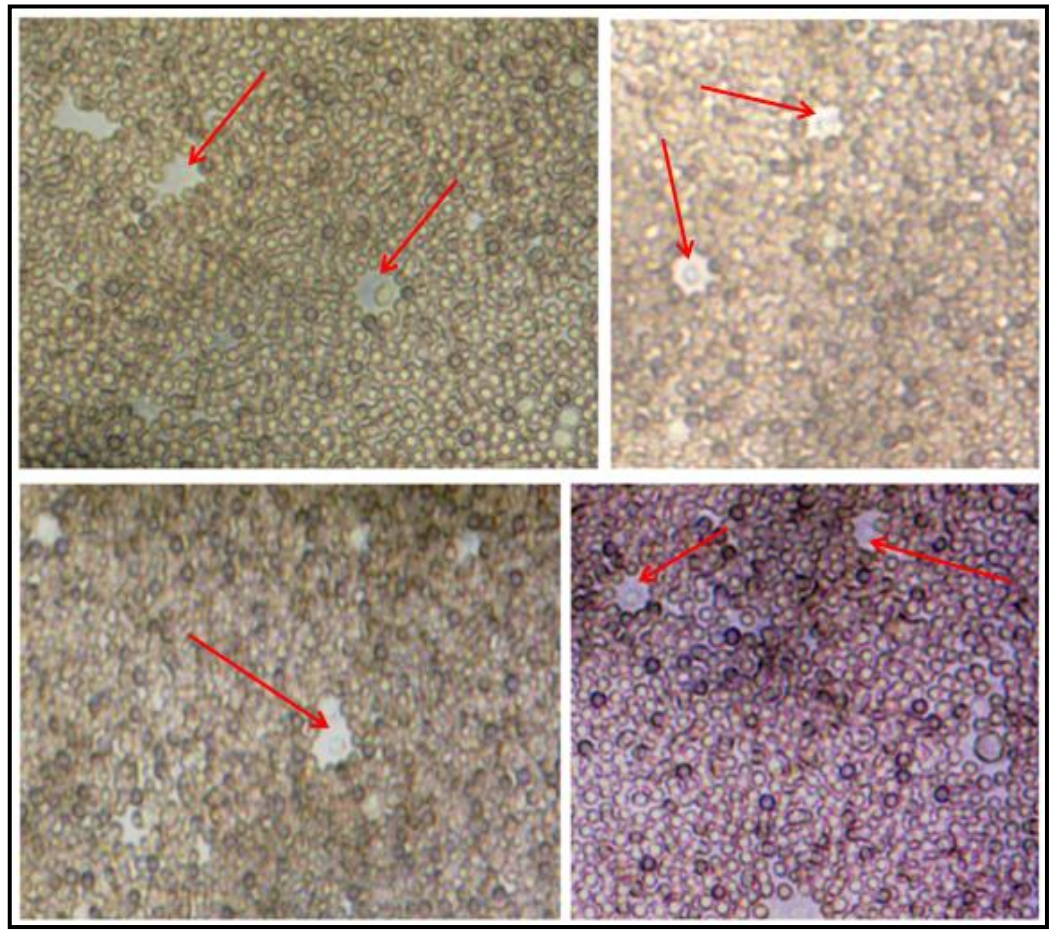

Figure: Images of PFC assay (Red arrow indicate "plaques")

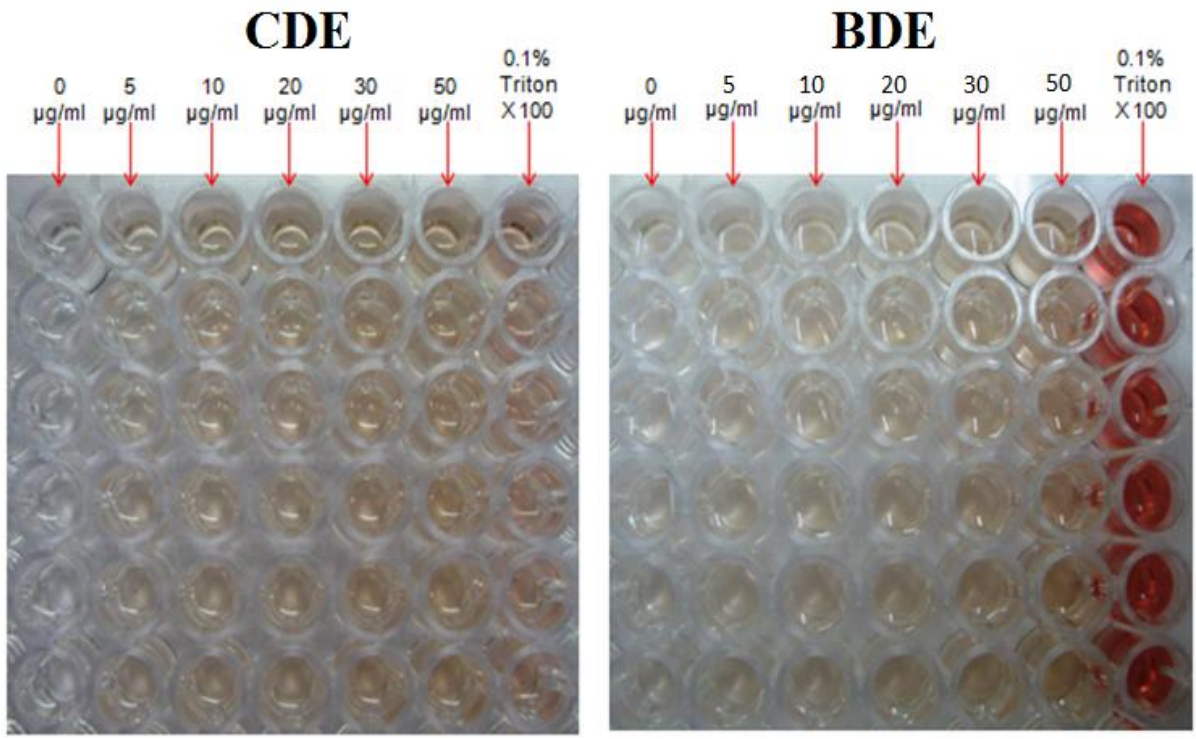

Figure: Dose-dependent increase in the optical density (O.D) of the sample in the microtitre plate. Increase in the O.D indicates liberation of more hemoglobin from the erythrocytes in the suspending medium, hence more the color. Therefore, the above experiment indicates that both CDE and BDE cause hemolysis.

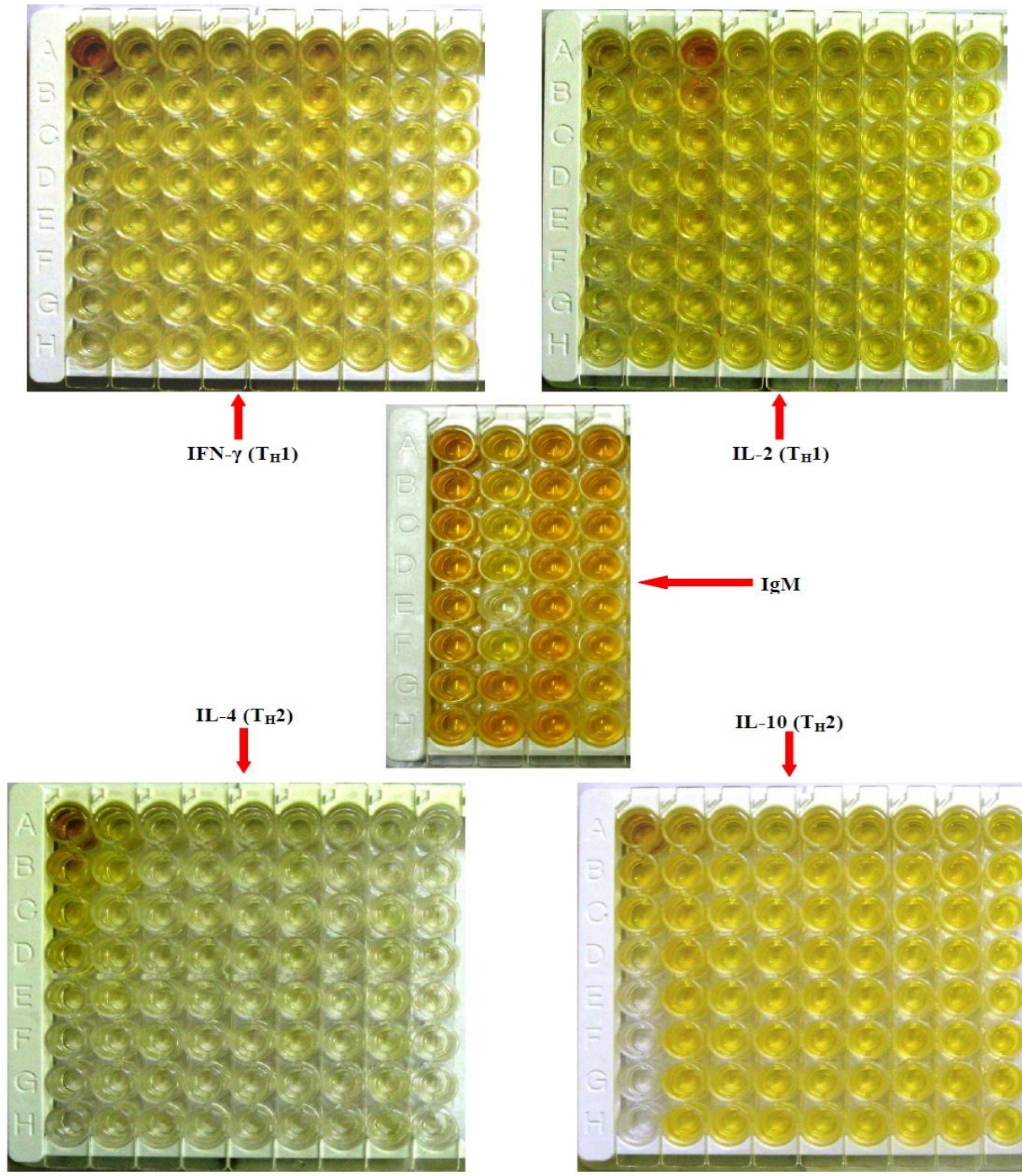

Figure: Photographs of ELISA plates of different TH1 and TH2 cytokines and Immunoglobulin M (IgM)

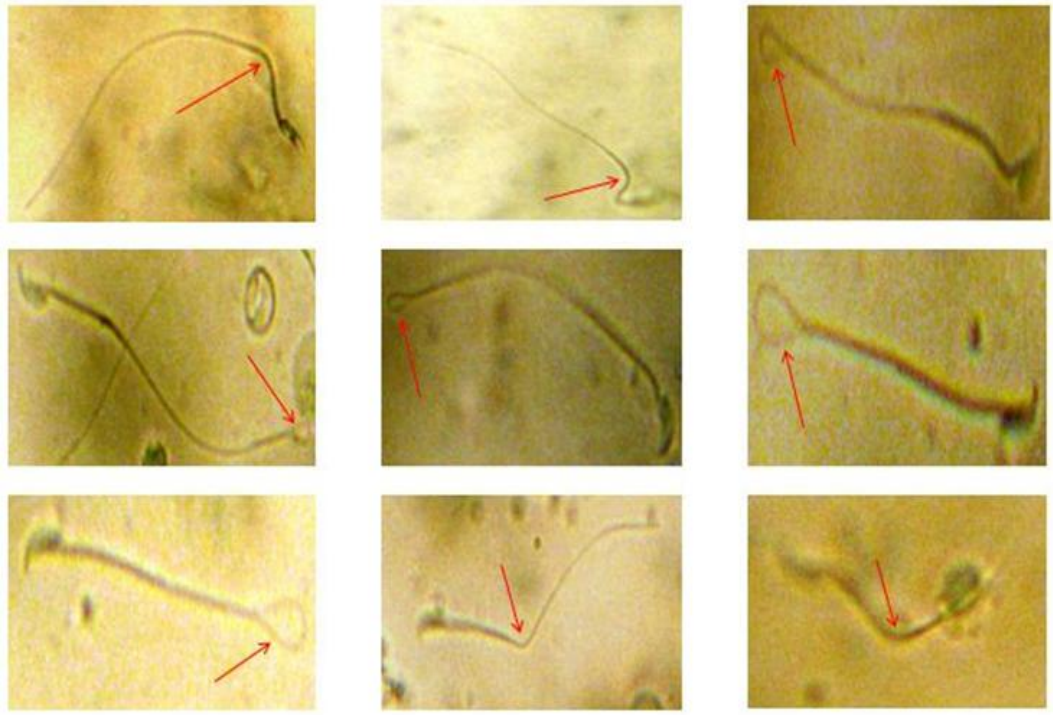

Figure: Some active spermatozoa (indicated by red arrow) showing coiling during HOST

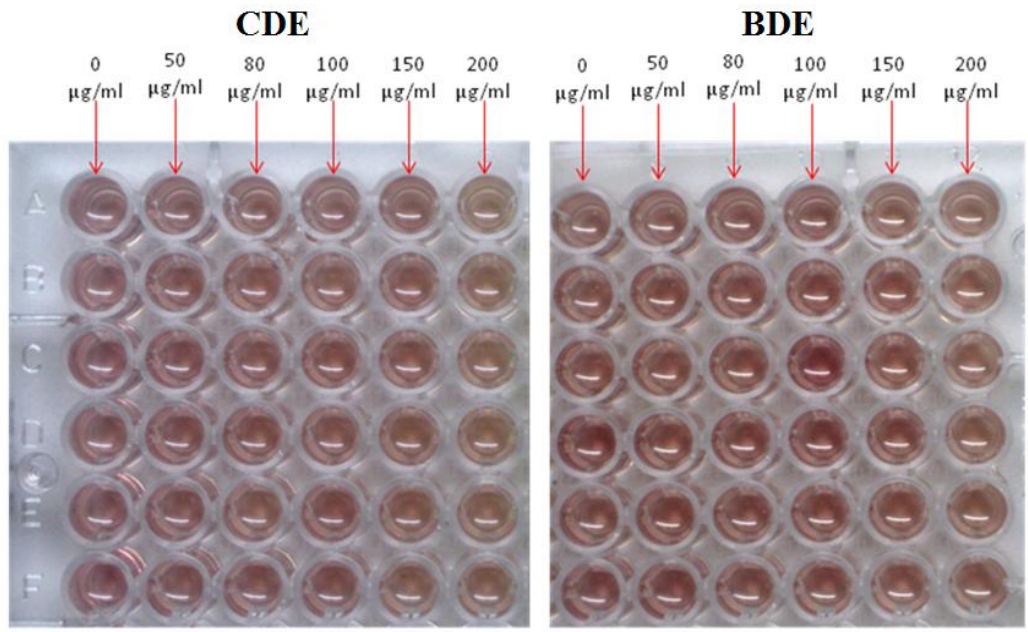

Figure: Photograph of sperm MTT assay after solubilization of formazan

APPENDIX – 3

PUBLICATIONS

1. **Roy S**, Chaudhuri TK. (2016). Toxicological assessment of *Diplazium esculentum* on the reproductive functions of male Swiss albino mouse. Drug and Chemical Toxicology, Published online 9 June 2016.

2. **Roy S**, Chaudhuri TK. (2015). Assessment of Th1 and Th2 cytokine modulatory activity of an edible fern, *Diplazium esculentum*. Food and Agricultural Immunology, 26: 690–702.

3. **Roy S**, Dutta S, Chaudhuri TK. (2015). In vitro assessment of anticholinesterase and NADH oxidase inhibitory activities of an edible fern, *Diplazium esculentum*. Journal of Basic and Clinical Physiology and Pharmacology, 26: 395–401.

4. **Roy S**, Tamang S, Dey P, Chaudhuri TK. (2013). Assessment of the immunosuppressive and hemolytic activities of an edible fern, *Diplazium esculentum*. Immunopharmacology and Immunotoxicology, 35: 365–372.

5. **Roy S**, Hazra B, Mandal N, Chaudhuri TK. (2013). Assessment of the antioxidant and free radical scavenging activities of methanolic extract of *Diplazium esculentum*. International Journal of Food Properties, 16: 1351–1370.

6. **Roy S**, Tamang S, Chaudhuri TK. (2013). Sperm viability assessment using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay of Swiss albino mice treated with *Diplazium esculentum*. Asian Journal of Pharmaceutical and Health Sciences, 3: 684–689.