Materials List for:

ELIME (Enzyme Linked Immuno Magnetic Electrochemical) Method for Mycotoxin Detection

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Materials

Name	Company	Catalog Number	Comments
Potassium chloride	Sigma-Aldrich	P9333	
Potassium dihydrogen phosphate	Sigma-Aldrich	P9791	
Disodium hydrogen phosphate	Sigma-Aldrich	S3264	
Sodium chloride	Sigma-Aldrich	S3014	
$MgCl_2$ anhydrous	Sigma-Aldrich	M8266	
DEA (99.5%)	Sigma-Aldrich	31589	
HCl 37%	Sigma-Aldrich	320331	
H_3BO_3	Sigma-Aldrich	B6768	
Tris[hydroxymethyl]-aminomethane	Sigma-Aldrich	252859	
BSA (albumin bovine serum)	Sigma-Aldrich	A4503	
NaOH	Sigma-Aldrich	S5881	
TWEEN 20	Sigma-Aldrich	P9416	
NaN_3	Aldrich	71290	
1-Naphthyl phosphate disodium salt	Fluka	N7255	
Skimmed milk blocking solution - non fat dry milk	Bio-Rad	170-6404	
HT-2 conjugated with KLH (Keyhole Limpet Hemocyanin), stock solution (1 mg/ml in PBS):	Biopure	004050	The HT-2-KLH conjugate was obtained by CDI-method where the free OH-groups on position 3 and 4 at the HT-2 toxin were activated by N,N'-carbonyldiimidazole (CDI) and the activated HT-2 toxin was let to react with aminogroups of the protein (KLH) to generate a stable carbammate linkage.
Secondary labeled antibody: Anti-mouse IgG (H+L) from horse, conjugated with Alkaline Phosphatase, concentration 1 mg/ml.	Vector Laboratories	AP-2000	
HT-2 toxin	Biopure		
Magnetic beads: Dynabeads®	Invitrogen	M-280 Tosylactivated	Concentration 2 x 10^9 beads/ml.
Phosphate buffered saline (PBS), pH 7.4			Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogen phosphate, 1.16 g of disodium hydrogen phosphate and 8.00 g of sodium chloride in 900 ml of water

Diethanolamine buffer, DEA, 0.97 M + 1 mM $MgCl_2$ + 0.15 M KCl, pH 9.8			Dissolve 0.0476g of $MgCl_2$ anhydrous and 7.3.59 g of KCl in ~ 300 ml of water. After dissolution add 51 ml of DEA (99.5%). Adjust pH to 9.8 with HCl (6 M). Dilute to 500 ml with water.
Borate buffer, 0.1 M, pH 9.5			Dissolve 3.09 g of H_3BO_3 in ~ 300 ml of distilled water; adjust pH to 9.5 with NaOH and/or HCl (6 M or lower concentrations). Dilute to 500 ml with distilled water.
TRIS buffer, 0.2 M, pH 8.5			Dissolve 3.85 g of Tris[hydroxymethyl]-aminomethane in 100 ml of water. Adjust to pH 8.5 with NaOH and/or HCl (6 M or lower concentrations). Dilute to 200 ml with water.
TRIS buffer + BSA solution (0.1%), pH 8.4			Dissolve 0.050 g of BSA in 50 ml of TRIS buffer pH 8.4. This solution must be freshly prepared on the day of use.
PBS buffer + TWEEN® 0.05%			Dissolve 0.250 g of TWEEN 20 in 500 ml of the previously prepared PBS buffer, pH 7.4
PBS buffer + BSA solution 0.1%			Dissolve 0.050 g of BSA in 50 ml of PBS buffer, pH 7.3. This solution must be freshly prepared on the day of use.
PBS buffer + BSA 0.1% + NaN_3 0.02%			Dissolve 0.050 g of BSA and 0.010 of NaN_3 in 50 ml of PBS buffer, pH 7.3. Storage solution
Enzymatic substrate			Dissolve 0.010 g of 1-Naphthyl phosphate sodium salt in 100 ml of DEA buffer pH 9.8. Wrap the flask tightly in aluminium foil. This solution must be freshly prepared on the day of use.
Skimmed milk blocking solution			Add 0.20 g of Blotting Grade Blocker Non-Fat Dry Milk (Bio-Rad, Hercules, CA, USA) to 200 ml of PBS buffer pH 7.3. This solution must be freshly prepared on the day of use.
HT-2 stock solution			Dissolve 10 mg of trichothecene HT-2 toxin vial, in 10 ml of acetonitrile to give a solution with a concentration of 1 mg/ml. Split up this solution into single-use aliquots of 30 ml and store them at less than -30 °C.
HT-2 Working standard solution			Pipette 20 ml of HT-2 toxin stock solution into a 2 ml calibrated volumetric flask and dilute with acetonitrile to obtain a HT-2 working solution containing 10 µg /ml of trichothecene toxin. This solution should be freshly prepared on the day of use.
HT-2 Working calibrant solutions			Dilute the HT-2 working standard solution (10 µg/ml) to prepare working calibrant solution 100 ng/ml. Dilute HT-2 working calibrant solution 100 ng/ml to prepare

			working calibrant solutions of the following concentrations 0 (blank), 0.5, 1, 2, 4, 10 ng/ml. These solutions must be prepared fresh on the day of use.
HT-2 conjugated with KLH			Split up HT-2 conjugated with KLH (Keyhole Limpet Hemocyanin) stock solution (1 mg/ml in PBS) into single-use aliquots of 350 µl. Store aliquots at -30 °C.
Specific Monoclonal Antibody			Dilute 1:350 (v:v) the stock concentration (1.383 mg/ml) of monoclonal antibody in PBS to use in competition step. This solution must be prepared fresh at the moment of use. 10 ml antibody stock solution in PBS for a final volume of 3.5 ml, are enough to analyze 17 standards and /or samples.
Secondary Labeled Antibody			Anti-mouse IgG (H+L) conjugated with Alkaline Phosphatase is diluted 1:100 (v:v) in PBS to use in competition step. 100 µl antibody stock solution in PBS for a final volume of 10 ml, are enough to analyze 25 standards and /or samples. This solution must be prepared fresh at the moment of use.
Magnetic Particle Concentrator: MPC®-S	Invitrogen		
PalmSens instrument	PalmSens		Provided with PalmSens Lite, Serial cable connecting PC laptop and Mux options software
CH8 PalmSens Multiplexer	PalmSens		
Eight channel Mux electric contact			hand made
Strip with eight screen printed electrodes (SPEs)			hand made
Specially designed support for electrodes strip			hand made. Includes 8 neodymium magnets each of which is placed below each working electrode surface of the SPE
Calibrated microliter pipettes	Gilson, Inc.		
Magnetic stirrer and stir bars.			
Glass beakers (100, 50, 20 ml).			
Volumetric flasks (2ml).			
0.2 and 2ml Eppendorf tubes.	Eppendorf		
Falcon tubes of 5ml and 15ml.	Falcon BD		
Laboratory Vortex Mixer			Do not use vortex mixer to resuspend magnetic beads coated with HT2-KLH or linked with immunological chain to avoid denaturing of proteic parts
Laboratory oven or thermostated room			Choose a oven able to keep a temperature of 37±3 °C.