

KTL-510 peptide ELISA protocol for detection of anti-enterovirus IgG

1. COATING

- dilute KTL-510 stock solution (1 mg/ml) in TRIS-buffer pH 9.0 (0.01 M) - final dilution 5 µg/ml
- add 100 µl KTL-510 (5µg/ml) per well
- incubation overnight (to 5 days) at 8°C

2. WASH

- 3x washing with PBS containing 0.1% Tween 20

3. BLOCKING

- add 275 µl blocking solution (PBS with 0.1% BSA) per well
- incubation for 30 minutes at room temperature

4. WASH

- 3x washing with PBS containing 0.1% Tween 20

5. SAMPLES

- dilution of inactivated (30 minutes at 56°C) sera samples in PFTM buffer (dilutions 1:100; 1:1000; 1:10000)
- add 100 µl of diluted sample per well (in parallels)
- incubation for 1 hour at 36°C

6. WASH

- 3x washing with PBS containing 0.1% Tween 20

7. CONJUGATE

- dilute polyclonal rabbit Anti-human-IgG-HRP (DAKO P0214) antibody in PFTM solution (1:6000)
- add 100 µl per well
- incubation for 1 hour at 36°C

8. WASH

- 3x washing with PBS containing 0.1% Tween 20

9. SUBSTRATE

- substrate –UP-buffer (containing urea-peroxide, sodium citrate, sodium acetate trihydrate) pH 5.5, distilled H₂O and 3,3',5,5'-Tetramethylbenzidine (6 mg in 1ml DMSO)
- add 100 µl per well
- incubation 10 minutes at room temperature

10. STOP REACTION

- add 100 µl H₂SO₄ per well

11. READING

- at 450 nm

Preparation of solutions

Phosphate Buffered Saline (PBS) pH 7.2

NaCl	40g
KCl	1g
KH ₂ PO ₄	1g
Na ₂ HPO ₄ x 12H ₂ O	14.505g

- dissolve in 5l distilled water

TRIS buffer pH 9.0 (0.01M)

Tris(hydroxymethyl)aminomethane	2.42g
NaCl	17.54g
EDTA	1.16g

- dissolve in 2l distilled water

Peptide KTL-510 (GenScript)

- Sequence: KEVPALTAVETGATC
- preparation of stock solution - 1 mg/ml in distilled water
- dilute the stock solution in TRIS buffer pH 9.0 (0.01M) to 5µg/ml (before use!)

0.1% BSA in PBS (blocking reagent)

Bovine Serum Albumin (powder)	0.2g
PBS	200ml

PFTM solution (dilution buffer)

PBS	
Fetal Bovine Serum	1%
Bovine Serum Albumin (powder)	1%
Tween 20	0.1%
EDTA	5mM

UP-buffer

Citric acid monohydrate	10g
NaAc.3H ₂ O	204g
<ul style="list-style-type: none"> dissolve in 1.5L distilled water pH measurement - 5.45 - 5.55 (adjust with Citric acid monohydrate) to 1428L of such a solution add: 	
hydrogen peroxide urea	2g
<ul style="list-style-type: none"> filter through 0.22µ filter store at 4°C 	

3,3',5,5'-Tetramethylbenzidine (TMB)

- dissolve 240mg TMB in 40ml DMSO (6mg/ml)

Substrate

UP-buffer	4.25ml
distilled H ₂ O	38.5ml
TMB	0.55ml