



“Do-It-Yourself” (DIY) ELISA for HIV-1-p24 Antigen

Buffers

- Coating buffer** 100mM NaHCO₃ pH 8.5
8.4g in 1L = pH 8.0
≈450µl 10M NaOH in 1L gives pH 8.5
- 10xTBS** 1.44M NaCl, 0.5% Tween 20, 250mM Tris pH 7.5
168.32g NaCl in 2L
10ml neat Tween 20 in 2L
60.6g Tris in 2L gives pH 10.1
≈30ml Conc. HCL in 2L gives pH 7.5
- Blocking buffer** 0.4g milk powder in 20ml 1xTBS
2g milk powder in 100ml enough for 4-5 plates
- PBS / E / S** PBS with 0.1% empigen and 10% lamb serum
9ml PBS, 1ml serum, 70µl empigen for 10ml
- TMT / SS** 1xTBS with 2% milk, 20% lamb serum, 0.05% Tween 20

	25ml	50ml	100ml	150ml	200ml
Water (ml)	16.25	32.5	65	97.5	130
10xTBS (ml)	2.5	5	10	15	20
Milk (g)	0.5	1	2	3	4
Serum (ml)	5	10	20	30	40
Tween 20 (ml; 10% stock)	1.25	2.5	5	7.5	10

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aalto-bioreagents.ie Web: www.aalto-bioreagents.ie





6. **Reaction buffer** 10mM ethanolamine, 0.5mM MgCl₂, pH 9.8

0.61ml ethanolamine in 1L
0.5ml MgCl₂ (1M stock) in 1L gives pH 10.2
≈160µl Conc. HCl gives pH 9.8

ELISA REAGENTS

Item	Catalogue No.	Company	Units
Coating anti-HIV-1-p24	D7320	Aalto	2mg (2ml)
Biotin anti-HIV-1-p24*	BC 1071-BIOT*	Aalto	100µl
Streptavidin-AP	1089 161	Boehringer Mannheim	1ml, 1000U
pNPP substrate	S0942	Sigma	5mg tablets

* A suitable method of medium-term storage after receipt is to dilute 0.1ml of the biotinylated conjugate to 1.0ml (1:10) with TMT / SS buffer. Then aliquot and store 0.1ml volumes (sufficient for 1 plate) at -20°C. Prior to use, dilute 0.1ml to 10ml (1:100) in TMT / SS buffer.

Protocol

Coating the plate

Add 100µl of D7320 (diluted to 1mg/ml) to 9.9ml coating buffer and add 100µl to each well. Cover and leave overnight at room temperature.

Wash

Wash with 1xTBS for 3x200µl

Block

Add 200µl of blocking buffer to each well for 30 minutes

Wash

Wash with 1xTBS for 3x200µl

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aaltoioreagents.ie Web: www.aaltoioreagents.ie



1.8. EN ISO 9001:2000





Add sample

Place 100µl of supernatant (previously treated with 0.1% - 0.2% emipigen and heat-inactivated for 30 minutes at 56°C) into well. For ELISA controls either add duplicate 100ng/ml p24 Ag standards (1:10 dilution of aliquot in PBS / E / S) or use a standard curve; 46.25µl transfer volume in 100µl ($46.25 / 146.25 = 316$) to give 1000, 316, 100, 31.6, 10, 3.16 and 1ng/ml. Use PBS / E / S as negative controls. Cover and leave overnight at room temperature.

Wash

Wash with 1xTBS for 6x200µl

Biotinylated antibody binding

Dilute 0.1ml of stock solution of biotinylated antibody conjugate to 10ml (1:100) in TMT / SS. Then add 100µl per well and leave at room temperature for 2 hours.

Wash

Wash with 1xTBS for 6x200µl

Streptavidin binding

Dilute 20µl of streptavidin-AP reagent in 10ml TMT / SS. Add 100µl to each well and leave at room temperature for 1 hour.

Wash

Wash with 1xTBS for 6x200µl

Substrate

Add 100µl (2x5mg tablets in 10ml reaction buffer) pNPP solution to each well. Place 100µl of same in first column off a blanking plate. Incubate in the dark until the O.D. of 100ng/ml control >1.000. Read at 405nm wavelength.

Aalto Bio Reagents Ltd.

Church Lane, Rathfarnham Village
Dublin 14, Ireland.

Tel: +353-1-490 0685 Fax: +353-1-490 0122

E mail: info@aaltoioreagents.ie Web: www.aaltoioreagents.ie



I.S. EN ISO 9001:2000

