NEONATAL 17-OHP
Screening Elisa assay

Enzyme immunoassay for the titration of 17 Alpha-hydroxyprogesterone in newborns

Using DRIED BLOOD SPOTS

CE

ZenTech

ORDERING INFORMATION

*Code : **E-GQ-192***
Package Size : 192 tests/kit

*Code : **E-GQ-1920***
Package size : 1920 tests/kit

Indications

- Quantitative determination of 17 Alpha-Hydroxyprogesterone in blood samples dried on Schleicher & Schuell 903 filter paper.
- This kit is particularly suitable for screening of congenital hyperplasia (CAH) in newborns.

Features

- Convenient transport and good stability of the samples
- Accurate, sensitive and specific assay
- Non-isotopic method

Performance characteristics

- Detection limit : 1.82 ng/mL
- Measuring range : from 0 to 250 ng/mL

Kit contents

Reagents	Quantity 192 tests	Quantity 1920 tests
Microplates (breakable strips)	2 x 96	20 x 96
Calibrators 1-8	1 aluminium pouch	3 aluminium pouches
17-OHP conjugate	1 x 0.45 ml	10 x 0.45 ml
Extraction buffer	1 x 50 ml	1 x 500 ml
Controls	1 aluminium bag	5 aluminium bags
Washing Solution	1 x 55 ml	1 x 500 ml
Chromogenic substrate	2 x 11 ml	1 x 220 ml
Blocking Reagent	2 x 15 ml	1 x 250 ml

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Principle of the test

The 17-OHP Screening ELISA is an enzyme immunoassay to quantitate human 17 Alpha-Hydroxy-progesterone (17-OHP) using dried blood spots.

The 17-OHP Screening ELISA is a competitive ELISA. The strips are coated with an anti-17-OHP antibody that capture 17-OHP present in the sample. Dried blood spots are incubated with the extraction buffer on a shaker for 1 hour at room temperature prior to overnight incubation at 4 °C. The extraction buffer contains a 17-OHP molecule conjugated to horseradish peroxidase (HRP). The 17-OHP peroxidase conjugate is allowed to compete with 17-OHP present in the sample. The following complexes are formed :

Rabbit anti-17-OHP * 17-OHP in sample ◄► Rabbit anti-17-OHP * 17-OHP peroxidase conjugate

After a washing step, the immunocomplex is detected by reduction of 3.3'-5.5'-tetramethylbenzidine (TMB) by HRP. The development of a blue colour is inversely proportional to the amount of antigen in the sample or calibrator. The enzymatic reaction is stopped by addition of sulfuric acid and absorbance at 450 nm is read using an ELISA microtiter plate reader.

Analytical performances

Precision

The intra-assay imprecision has been tested with 2 samples containing various amounts of 17-OHP in 8 replicates.

	17-OHP (ng/ml)	Standard Deviation	CV (%)
Sample 1	7.49	0.809	10.8
Sample 2	52.84	4.558	8.63

The inter-assay precision has been tested in 3 separated assays with 2 samples containing various amounts of 17-OHP, each sample tested in 8 replicates.

	17-OHP (ng/ml)	Standard Deviation	CV (%)
Sample 1	7.8	0.3	3.4
Sample 2	55.8	11.6	20.8

Recovery

Known amounts of 17-OHP were added to sample to determine recovery of the assay.

	17-OHP Added (ng/ml)	17-OHP Recovered (ng/ml)	Recovery (%)
Sample 1	200	213.98	107
	100	109.96	110
	25	23.06	92.2
	50	55.11	110.2

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