



AccuDiag™

Pro-GRP (Pro-gastrin-releasing peptide)

ELISA Kit

REF 7015

PIC TM7015VU1

IVD See External Label 2°C 96 Tests

Pro-GRP ELISA

Method	Enzyme Linked Immunosorbent Assay
Sample	50 µl
Range	0-1000 pg/ml
Cut-off value	46 pg/ml
Incubation Time	120 minutes
Shelf Life	12-14 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The DAI Pro-GRP ELISA is a quantitative enzyme immunoassay for the *in vitro* detection of pro-gastrin-releasing peptide (ProGRP 31-98) in human serum or plasma. This assay has the advantage of being sensitive, specific, simple and fast. It has great significance in the diagnosis of small cell lung cancer (SCLC).

MATERIALS AND COMPONENTS

Materials provided with the test kit

Description	Quantity
Microplate	96 wells
POD-Conjugate	3 bottles

Standard ProGRP	2 bottles
Standard Dilution Buffer	20 mL X 1
Reaction Buffer	20 mL X 1
Conjugate Buffer	20 mL X 1
Concentrated Wash Buffer	100 mL X 1
OPD Substrate tablet	4 tablets
Substrate Buffer	20 mL X 1
Stop Solution	20 mL X 1
Plate Cover	3 pieces
Pincette (for picking up substrate tablet)	1
Insert	1 copy

PREPARATION OF SOLUTIONS

Solution	Preparation	Stability
POD-Conjugate Solution	Dissolve contents of bottle 1 in 5mL conjugate Buffer from bottle 5	Two weeks at 2-8°C
Standard solution	Dissolve contents of bottle 2 in 1mL distilled water (= 1000 pg/mL)	One week at 2-8°C
Washing solution	Dilute contents of bottle 6 with distilled water to 10 times	One month at 2-8°C
Substrate solution prepared	Dissolve one tablet picked up from bottle 7 with plastic pincette in 5 mL substrate buffer from bottle 8	The solution should be prepared at least 10 minutes before using, and used within 3 hours. Use substrate solution after bubbles have disappeared.

Preparation of standard serial dilution

Mix standard solution and diluent from bottle 3 in the relationship 1+2 (=333-3 pg/ml). Repeat this procedure three times, then prepare five standard solutions. (1000, 333-3, 111-1, 37-0, 12-3 pg/ml). Use diluent as standard of zero concentration.

ASSAY PROCEDURE

- One well must be blank. Add 100 µl of reaction buffer from bottle 4, and then 50 µl of standard solution or samples into the other wells, and shake plate calmly.
- Cover the plate with plate cover, and incubate at 37°C for 60 minutes.
- Wash the plate: Aspirate contents from each well. Add 350 µl of washing solution into each well including blank well, and aspirate contents. Repeat this procedure 5 times.
- Add conjugate: Add 100 µl of POD-conjugate solution into each well (except the blank well). Cover and incubate for 30 minutes at room temperature.
- Wash the plate: Repeat the wash procedure as in step 3.
- Add 100 µl of Substrate Solution into each well including blank well. Cover and incubate for 30 minutes at room temperature.
- Add 10 µl stop solution into each well including blank well, mix well.
- Read the absorbance at 490-495 nm/600-700 nm of the wave length for less than 1 hour after stop reaction



CALCULATION OF RESULTS

1. Calculate mean of absorbance of ProGRP standard solution.
2. Plot the absorbance mean of standard solution on longitudinal axis, and concentrations of ProGRP standard solution on abscissa axis in both logarithms graph paper and make standard curve.
3. Calculate mean of sample absorbance.
4. Calculate concentration of ProGRP in samples using standard curve.
5. Concentration of ProGRP can be calculated also by least squares.
6. Dilute and retest the sample which absorbance is over the standard curve.

PERFORMANCE CHARACTERISTICS

1. Absorbance operated diluent (=pg/ml) as sample shows less than 0.1.
2. Measuring range: 0-1000 pg/ml
3. Cut off value: 46 pg/ml (see table 1)
4. Precision: CV in series shows within 15% (n=5)

Table 1: Sensitivity and specificity of various cut-off values

Cut off value (pg/ml)	Sensitivity (%)	Specificity (%)	
		(1)	(2)
20	86	60	59
25	81.5	76	75
31	76	87	87.5
35	72	90.5	91
40	68	94	94
46	65	96	96
50	64	96	97
55	63	97	97.5
60	62	98	98
65	61.5	98	98
70	60	98	99
75	59	98	99
80	58	99	99
85	57	99	99
90	57	99	99
95	56	99	99
100	55	99	99

STORAGE

1. Store all reagents at 2-8°C.
2. Avoid direct rays of the sun during the storage and reaction.

NOTICE

1. In case of preparation of substrate solution, use the clean equipment made of nonmetal.
2. Do not let metal apparatus touch stop solution.
3. Pay attention not to touch substrate tablet and stop solution. Wash away with water quickly if it is touched.
4. Carry out operation quickly, in particular enzyme reaction.
5. Make standard curve every assay.
6. Aspirate the solution completely in case of the lasting procedure.
7. Measure again, when each absorbance goes over range of +20% for mean of double determination.
8. Dispose used plate after autoclaving.

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485
Quality
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Medical Devices
CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

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REF 7015	AccuDiag™ - Pro-GRP ELISA
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EU REP	AR Experts BV, Boeingavenue 209, 1119 PD Schiphol-Rijk, The Netherlands info@ar-experts.eu
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