

**FOR INFORMATION ONLY.
WHEN PERFORMING
THE ASSAY ALWAYS REFER
TO PACKAGE INSERT
SUPPLIED
WITH THE KIT**



CanAg ProGRP EIA

Prod. No. 220-85














Instructions for use

Enzyme immunoassay kit

2007-09

For 96 determinations

For Research Use Only-Not for use in diagnostic procedures

 Use By	 Temperature limitation
 Batch code	 Consult Instructions for Use
 Date of manufacture	 Biological risks
 Catalogue number	 Contents of kit
 Manufacturer	 From mouse
 Contains sufficient for <96> tests	 From goat
	 Human origin

INTENDED USE

The CanAg ProGRP EIA kit is intended for the quantitative determination of ProGRP in serum.

SUMMARY AND EXPLANATION OF THE ASSAY

ProGRP is a stable precursor of the gut hormone GRP (Gastrin Releasing Peptide). GRP, originally isolated from porcine stomach, is secreted from Small Cell Lung Cancer (SCLC) cells. Although detection of serum GRP has been expected to be useful for diagnosis of SCLC, determination of serum GRP has not been feasible owing to its instability in blood. The ProGRP peptide however, is stable in serum and can be used as a serological marker for GRP. Serum levels of proGRP have been shown to be elevated in patients diagnosed with SCLC.

PRINCIPLE OF THE TEST

The CanAg ProGRP EIA is a solid-phase, one-step, non-competitive immunoassay based on antibodies specific for different epitopes specifically expressed in ProGRP. Calibrators, controls or unknown samples are incubated together with affinity purified biotinylated Anti-ProGRP polyclonal antibody and horseradish peroxidase (HRP) labelled Anti-ProGRP Monoclonal antibody E146 in Streptavidin coated microtiter strips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour is proportional to the amount of ProGRP present in the samples.

The colour intensity is determined in a microplate spectrophotometer at 450 nm after addition of Stop Solution. Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The ProGRP concentrations of unknown samples are then read from the calibration curve.

REAGENTS

- Each CanAg ProGRP EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8° C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2-8°C immediately after use.

Component	Quantity	Storage and stability after first use
-----------	----------	---------------------------------------

MICROPLA		
Streptavidin Microplate	1 Plate	2–8°C until expiry date stated on the plate
12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminium pouch, containing desiccant. Reseal carefully to keep dry.		

ProGRP Calibrators	6 vials, lyophilized	Stability after reconstitution 3 days at 2-8° C 2 months at -20° C or below
---------------------------	----------------------	---

CAL	ProGRP	A	1 x 1 mL
------------	---------------	----------	----------

CAL	ProGRP	B	1 x 1 mL
------------	---------------	----------	----------

CAL	ProGRP	C	1 x 1 mL
------------	---------------	----------	----------

CAL	ProGRP	D	1 x 1 mL
------------	---------------	----------	----------

CAL	ProGRP	E	1 x 1 mL
------------	---------------	----------	----------

CAL	ProGRP	F	1 x 1 mL
------------	---------------	----------	----------

The lyophilised calibrators contain cell line derived ProGRP in a protein matrix with an inert yellow dye and a non-azide preservative. To be reconstituted with 1 mL of distilled or deionised water before use.

NOTE: The exact ProGRP concentration is lot specific and is indicated on the label of each vial.

Component	Quantity	Storage and stability after first use
-----------	----------	---------------------------------------

STOP		
STOP Solution	1 x 15 mL	2–8°C until expiry date stated on the vial
Contains 0.12 M hydrochloric acid. Ready for use.		

WASHBUF	25X	
Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative. To be diluted with distilled or deionised water 25 times before use.		

Indications of instability

The TMB HRP-Substrate should be colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

FOR RESEARCH USE ONLY: THE PERFORMANCE CHARACTERISTICS OF THIS PRODUCT HAVE NOT BEEN ESTABLISHED

No clinical decision or patient notification should be made based on the results obtained with this product.

- Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88–8395 on laboratory safety procedures or any other local or national regulation.
- Handle all serum specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

Caution

Material used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV 1 and 2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

SPECIMEN COLLECTION AND HANDLING

The CanAg ProGRP EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Serum can be stored at 2–8°C for 24 hours, before being tested. For longer periods store samples at -40°C or colder.

Bring frozen samples to room temperature and mix THOROUGHLY by gently inverting multiple times before analysis. Samples that contain gross particulates should be centrifuged at 10,000 x g for 10 minutes, prior to use to eliminate any particulate matter that may have developed from the thawing process.

PROCEDURE

Materials required but not supplied with the kit

1. Microplate shaker

Shaking should be medium to vigorous, approximately 700-900 oscillations/min.

2. Microplate wash device

Automatic plate washer capable of performing 1 and 6 washing cycles, and with a minimal fill volume of 350 µL/well/washcycle.

An 8-channel pipette with disposable plastic tips for delivery of 350 µL is recommended if an automatic microplate washer is not used.

3. Microplate spectrophotometer

With a wavelength of 450 nm and an absorbance range of 0 to 3.0.

4. Precision pipettes

With disposable plastic tips for dispensing microlitre volumes. An 8-channel pipette or dispenser pipette with disposable plastic tips for delivery of 100 µL is recommended but not required. Pipettes for dispensing millilitre volumes.

5. Distilled or deionised water

For reconstitution of ProGRP Calibrators, ProGRP Controls and for preparation of diluted Wash Solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg ProGRP EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25°C) prior to use. Frozen specimens must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators and patient specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. The requirement for efficient and thorough washing for separation of bound and unbound antigen and reagents from the solid-phase bound antibody-antigen complexes is one of the most important steps in an EIA. In order to ensure efficient washing make sure that all wells are completely filled to the top

edge with wash solution during each wash cycle, that wash solution is dispensed at a good flow rate, that the aspiration of the wells between and after the wash cycles is complete and that the wells are empty. If there is liquid left, invert the plate and tap it carefully against absorbent paper.

Automatic strip washer: Follow the manufacturer's instructions for cleaning and maintenance diligently and wash the required number of wash cycles prior to and after each incubation step. The aspiration/wash device should not be left standing with the Wash Solution for long periods, as the needles may get clogged resulting in poor liquid delivery and aspiration.

5. The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial into a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or dispenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper precision pipetting technique when handling samples and reagents. Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over. A diligent pipetting technique is of particular importance when handling the samples and the TMB HRP-Substrate solution.

Preparation of reagents	Stability of prepared reagent
ProGRP Calibrators	3 days at 2–8°C 2 months at -20°C or below
Add exactly 1.0 mL of distilled or deionised water to each vial. Allow to stand for at least 15 minutes to reconstitute and mix gently before use. NOTE: The concentration of the calibrators is stated on the labels and should be used for calculation of results.	
ProGRP Controls	3 days at 2–8°C 2 months at -20°C or below
Add exactly 1.0 mL of distilled or deionised water to each vial and mix gently. Allow to stand for at least 15 minutes to reconstitute and mix gently before use. NOTE: The ranges of the controls are stated on the labels.	
Wash Solution	2 weeks at 2–25°C in a sealed container
Pour the 50 mL Wash Concentrate into a clean container and dilute 25-fold by adding 1200 mL of distilled or deionised water to give a buffered Wash Solution.	

Preparation of reagents	Stability of prepared reagent
--------------------------------	--------------------------------------

Antibody Solution

1 day at 2–8°C

Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-ProGRP with 1 mL of Biotin Anti-ProGRP per strip (see table below and the Protocol Sheet).

No. of Strips	Tracer, HRP Anti-ProGRP (µL)	Biotin Anti-ProGRP (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass tube for preparation of Antibody Solution.

NOTE: Do not prepare more Antibody solution than daily use.

ASSAY PROCEDURE

Perform each determination in duplicate for both calibrators, controls and unknown samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25°C) before use.

1. Start preparing ProGRP Calibrators, Controls, Wash Solution and Antibody solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.

NOTE: Pre-washing is essential, ensure that the wells are empty and start to add samples as soon as possible after washing.

3. Pipette 50 µL of each of the ProGRP Calibrators (CAL A, B, C, D, E, F), controls (C1, C2) or unknown samples (Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	Unk1				
B	Cal A	Cal E	Unk1				
C	Cal B	Cal F	Unk2				
D	Cal B	Cal F	Unk2				
E	Cal C	C1					
F	Cal C	C1					
G	Cal D	C2					
H	Cal D	C2					

4. Add 100 µL of Antibody solution to each well using an 8-channel 100 µL precision pipette. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid.
5. Incubate the frame containing the strips for 2 hours (\pm 10 min) at room temperature (20–25°C) constantly shaking the plate using a microplate shaker.
6. Wash each strip 6 times, using the wash procedure described in Procedural notes, item 4 (above).
7. Add 100 µL of TMB HRP-Substrate to each well using the same pipetting procedure as in item 4 (above). The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between the addition to the first and last well should not exceed 5 min.
8. Incubate for 30 min (\pm 5 min) at room temperature (20–25°C) with constant shaking. Avoid direct sunlight.
9. Add 100 µL of Stop Solution to each well. Mix and read absorbance at 450 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg ProGRP EIA measures concentrations between 5 and 1000 ng/L. If ProGRP concentrations above the measuring range are to be expected, it is recommended to dilute samples with Sample Diluent prior to analysis (see "Calculation of results with diluted samples").

Quality control

ProGRP Control 1 and 2 should be used for validation of the assay series. Ranges of expected results are indicated on the vial labels. If values outside of the specified range are obtained, a complete check of reagents and reader performance should be made and the analysis repeated. Each laboratory may also prepare its own serum pools at different levels, which can be used as internal controls in order to assure the precision of the assay.

Reference material

Since no common reference material is available for ProGRP antigen, CanAg ProGRP EIA Calibrator values are assigned against a set of in-house reference standards.

CALCULATION OF RESULTS

If a microplate spectrophotometer with built-in data calculation program is used, refer to the manual for the spectrophotometer and create a program using the concentration stated on the label of each of the ProGRP Calibrators.

For automatic calculation of ProGRP results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator A should be included in the curve with the value 0 pM.
- Interpolation with point-to-point evaluation. Calibrator A should be included in the curve with the value 0 pM.

NOTE: 4-parametric or Linear regression evaluation methods should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each ProGRP Calibrator against the corresponding ProGRP concentration (in ng/L).

The unknown PROGRP concentrations can then be read from the calibration curve using the mean absorbance value of each patient specimen.

If samples in an initial analysis give ProGRP levels higher than calibrator F, then the samples should be diluted 1/10 and 1/20 with ProGRP Sample Diluent to obtain the accurate ProGRP concentration of the samples. Make fresh dilutions before the run.

1/10 dilution = 50 µL of specimen + 450 µL of ProGRP Sample Diluent

1/20 dilution = 200 µL of 1/10 dilution + 200 µL of ProGRP Sample Diluent

The ProGRP concentration of the undiluted sample is then calculated as:

Dilution 1/10: 10 x measured value

Dilution 1/20: 20 x measured value

Protocol Sheet

ProGRP EIA REF 220-85

Prepare the components directly before use. Use wash and incubation conditions according to the Instructions.

Step	Vial/Plate	Procedure																																							
1. Prepare ProGRP Calibrators	CAL ProGRP	Add 1.0 mL of distilled or deionised water to each vial. Allow to stand for at least 15 minutes. Mix gently before use. NOTE: The exact concentration of each calibrator is stated on the label. Reconstituted stability: 3 days at 2-8°C. 2 months at -20°C or below.																																							
Prepare ProGRP Controls	CONTROL ProGRP A, B, C, D, E, F 1, 2																																								
Prepare Wash Solution	WASHBUF 25X																																								
Prepare Antibody Solution	CONJ Anti-ProGRP BIOTIN Anti-ProGRP																																								
		<table border="1"> <thead> <tr> <th>No. of Strips</th> <th>Tracer, HRP Anti-ProGRP (µL)</th> <th>Biotin Anti-ProGRP (mL)</th> </tr> </thead> <tbody> <tr><td>1</td><td>50</td><td>1</td></tr> <tr><td>2</td><td>100</td><td>2</td></tr> <tr><td>3</td><td>150</td><td>3</td></tr> <tr><td>4</td><td>200</td><td>4</td></tr> <tr><td>5</td><td>250</td><td>5</td></tr> <tr><td>6</td><td>300</td><td>6</td></tr> <tr><td>7</td><td>350</td><td>7</td></tr> <tr><td>8</td><td>400</td><td>8</td></tr> <tr><td>9</td><td>450</td><td>9</td></tr> <tr><td>10</td><td>500</td><td>10</td></tr> <tr><td>11</td><td>550</td><td>11</td></tr> <tr><td>12</td><td>600</td><td>12</td></tr> </tbody> </table>	No. of Strips	Tracer, HRP Anti-ProGRP (µL)	Biotin Anti-ProGRP (mL)	1	50	1	2	100	2	3	150	3	4	200	4	5	250	5	6	300	6	7	350	7	8	400	8	9	450	9	10	500	10	11	550	11	12	600	12
No. of Strips	Tracer, HRP Anti-ProGRP (µL)	Biotin Anti-ProGRP (mL)																																							
1	50	1																																							
2	100	2																																							
3	150	3																																							
4	200	4																																							
5	250	5																																							
6	300	6																																							
7	350	7																																							
8	400	8																																							
9	450	9																																							
10	500	10																																							
11	550	11																																							
12	600	12																																							
2. Wash	MICROPLA	Wash each well once with Wash Solution. Use manual or automatic washer.																																							
3. Add calibrators, controls and samples	CAL ProGRP A, B, C, D, E, F CONTROL ProGRP 1, 2	50 µL in each well																																							
4. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well																																							
5. Incubate	MICROPLA	2 hours (± 10 min) shaking at room temperature (20-25°C)																																							
6. Wash	MICROPLA	Wash each well six times with Wash Solution. Use manual or automatic washer.																																							
7. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well																																							
8. Incubate	MICROPLA	30 min (± 5 min) shaking at room temperature (20-25°C)																																							
9. Add Stop Solution	STOP	100 µL in each well																																							
10. Incubate	MICROPLA	1 min shaking at room temperature																																							
11. Read absorbance	MICROPLA	Read at 450 nm within 15 min																																							



CanAg[®] is a registered trademark of Fujirebio Diagnostics AB

Fujirebio Diagnostics AB
Majnabbeterminalen
SE-414 55 Göteborg
Sweden
Phone + 46 31-85 70 30
Fax + 46 31-85 70 40
info@fdab.com
www.fdab.com