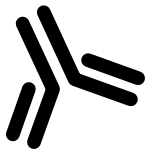


FOR INFORMATION ONLY.  
WHEN PERFORMING  
THE ASSAY ALWAYS REFER  
TO PACKAGE INSERT  
SUPPLIED  
WITH THE KIT



# CanAg ProGRP EIA

REF

220-10

IVD



Instructions for use. 2011-09

EN	EXPLANATION OF SYMBOLS
BG	ОБЯСНЕНИЕ НА СИМВОЛИТЕ
CS	VÝZNAM SYMBOLŮ
DA	SYMBOLFORKLARING
DE	ERKLÄRUNG DER SYMBOLE
EL	ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
ES	SIGNIFICADO DE LOS SÍMBOLOS
ET	SÜMBOLITE SELGITUS
FR	EXPLICATION DES SYMBOLES
HR	OBJAŠNJENJE SIMBOLA
HU	JELMAGYARÁZAT
IT	SPIEGAZIONE DEI SIMBOLI
LT	SIMBOLIŲ PAAIŠKINIMAI
LV	SIMBOLU SKAIDROJUMS
NL	VERKLARING DER SYMBOLEN
NO	SYMBOLFORKLARING
PL	OBJAŚNIENIE SYMBOLI
PT	EXPLICAÇÃO DOS SÍMBOLOS
RO	SEMNIȚAȚIA SIMBOLURILOR
RU	ОБОЗНАЧЕНИЯ
SE	SYMBOLFÖRKLARING
SK	VÝZNAM SYMBOLOV
SL	RAZLAGA SIMBOLOV
SR	OBJAŠNJENJE SIMBOLA
TR	SEMBOLLERİN AÇIKLAMALARI



Use By/Годно до/Použitelné do/  
Holdbar til/Verwendbar bis/  
Ημερομηνία λήξης/Fecha  
de caducidad/Kölblük kuni/  
Utiliser jusque/Rok valjanosti/  
Felhasználható/Utilizzare entro/  
Sunautoti iki/Izletot līdz/Houdbaar  
tot/Brukes innen/Użyç przed/  
Prazo de validade/Expiră la/  
Использовать до/Använd före/  
Použite né do/ Uporabno do/  
Upotrebljivo do/Son Kullanna Tarihi

LOT

Batch code/Номер на партида/  
Číslo šarže/Lotnummer/  
Chargenbezeichnung/Αριθμός  
Παρτίδας/Código de lote/Partii  
kood/Code du lot/Kod serije/  
Sarzsám/Codice del lotto/  
Partijos kodus/Partijas kods/Lot  
nummer/Partikode/Kod partii/  
Código do lote/Număr de lot/  
Номер лота/Lotnummer/Číslo  
šarže/Številka serije/Kod partije/  
Parti Kodu



Date of manufacture/Дата на производство/Datum výroby/  
Produktionsdato/Herstellungsdatum/  
Ημερομηνία παραγωγής/Fecha de fabricación/Valmistamise kuupäev/  
Date de fabrication/Datum proizvodnje/  
Gyártási idő/Data di produzione/  
Pagaminimo data/Ražošanas datums/  
Productiedatum/Fremstillingsdato/  
Data produkcji/Data de fabrico/Data fabricației/Дата производства/  
Tillverkningsdatum/Dátum výroby/Datum izdelave/Datum proizvodnje/Üretim tarihi



Temperature limitation/  
Температурни граници/  
Теплотни омеzeи/  
Temperaturbegrænsning/  
Temperaturbegrenzung/  
Περιορισμοί θερμοκρασίας/  
Limites de temperatura/  
Temperatuuri piirang/  
Limite de température/  
Temperaturno ograničenje/  
Hőmérsékletre vonatkozó korlátozás/  
Limiti di temperatura/  
Temperatūriņai apribojimai/  
Temperatūras ierobežojums/  
Temperaturbepèrking/  
Temperaturbegrensninger/  
Temperaturey granične/  
Limite de temperatura/  
Limite de temperatură/  
Температурный режим/  
Temperaturbegrænsning/  
Теплотне обмеzenie  
Omejitve temperature/  
Temperaturno ograničenje/  
Sıcaklık sınırlaması/

**IVD**

In Vitro Diagnostic Medical Device/  
Медицински уред за диагностика  
ин vitro/Diagnostický zdravotnícký  
prostředek in vitro/Medicinsk udstyr til  
in vitro-diagnostik/In-vitro-Diagnostikum/  
Ιατροτεχνολογικό προϊόν για διάγνωση  
In Vitro/Dispositivo médico para  
diagnóstico in vitro/In vitro diagnostiline  
meditsiiniseade/Dispositif médical de  
diagnostic in vitro/Diagnostički medicinski  
uređaj In Vitro/In vitro orvosdiagnostikai  
eszköz/Dispositivo medico per test  
diagnostici in vitro/In Vitro Diagnostinė  
Medicinos Priemonė/Medicínska ierice  
in vitro diagnostikai/In vitro-diagnostisch  
medisch instrument/In vitro diagnostisk  
medisinsk utstyr/Wyrób medyczny do  
diagnostyki in vitro/Dispositivo Médico  
de Diagnóstico In Vitro/Dispozitiv medical  
pentru diagnostic in vitro/Только для  
диагностики In Vitro/Endast för in  
vitro-diagnostik/ Zdravotnícka pomôcka na  
diagnostiku in vitro/In vitro diagnostični  
pripomoček/Diagnostički medicinski  
uređaj In Vitro/<96> testleri için yeterlilik  
içerir



Contains sufficient for <96> tests/Съдържа  
достатъчно количество за тестове  
<96>/Lze použít pro <96> testů/Ineholder  
tilstrækkeligt/Inhalt ausreichend für <96>  
Prüfungen/Περεχόμενο επαρκές για  
«96» εξετάσεις/Contenido suficiente para  
<96> ensayos/Kogusest piisab <96> testi  
lääbiviimiseks/Contenu suffisant pour «96»  
tests/Sadržaj dovoljno za <96> testova/A  
doboz tartalma <96> vizsgálat elvégzéséhez  
elegendő/Contenuto sufficiente per «96»  
saggi/Turiny's skirtas alikti <96> tyrimus/  
Satur's pietiekams <96> testiem/Inhoud  
voldoende voor «96» testen/til «96» test/  
Tilstrækkelig innhold for <96> prøver/  
Wystarczy na wykonanie <96> testów/  
Conteúdo suficiente para «96» ensaios/  
Conținut suficient pentru 96 de teste/  
Содержит достаточные количества для  
«96» определений/Innehåller tillräckligt  
till «96» antal tester/Obsah postačuje na  
tento počet testov: <96>/Vsebinsa zadostuje  
za <96> testov/Sadržina dovoljna za <96>  
testova/<96> testleri için yeterlilik içerir

**REF**

Catalogue number/Каталожен номер/  
Katalogové číslo/Katalognummer/  
Bestellnummer/Αριθμός καταλόγου/  
Número de catálogo/Katalogoi number/  
Numéro de catalogue/Kataloški broj/  
Katalógusszám/Numero di catalogo/  
Katalogo numeris/Numurs katalogā/  
Catalogusnummer/Katalognummer/  
Numer katalogowy/Número do catálogo/  
Număr de catalog/Номер по каталогу/  
Produktnummer/Katalógové číslo/  
Kataloška številka/Kataloški broj/  
Katalog numarası



Consult Instructions for Use/  
Прочетете инструкцията за  
употреба/Konzultujte s návodem  
k použití/Se brugsanvisning/Siehe  
Gebrauchsanweisung/Συμβουλευτείτε  
της Οδηγίες σχετικά με τη χρήση/  
Consulte las instrucciones de uso/  
Vt kasutusjuhendit/Consulter le mode  
d'emploi/Pročítajte upute za uporabu/  
Olvassa el a használati utasítást/  
Consultare le istruzioni per l'uso/Dél  
naudojimo žiūrėkite instrukcijas/Izlasiet  
lietošanas instrukciju/Raadpleeg de  
instructies voor gebruik/Les instruksene  
for bruk/Sprawdzić w instrukcji użycia/  
Consulte as Instruções de Utilização/  
Consultați instrucțiunile de utilizare/  
Обратитесь к инструкции по  
применению/Se bruksanvisning/  
Prečítajte si návod na používanie/  
Pročítajte uputstvo za upotrebu/  
Kullanım Talimatlarını Bakınız



Contents of kit/Съдържание на набора/  
Obsah soupravy/Kittets indhold/Inhalt  
des Kits/Περιεχόμενα του κιτ/Contenido  
del kit/Komplekt sisaldab/Contenu du  
kit/Sadržaj opreme/A készlet tartalma/  
Contenuto del kit/Rinkinio turinys/  
Komplekta saturs/Inhoud van de set/  
Settets innhold/Zawartość zestawu/  
Conteúdo do kit/Conținutul setului/  
Компоненты набора/Kit innehåll/  
Obsah súpravy/Vsebina kompleta/Sadržaj  
opreme/Kitin içindekiler



Biological risks/Биологическа  
опасност/Biológická rizika/Biologisk  
fare/Biologische Gefahren/Βιολογικοί  
κίνδυνοι/Riesgos biológicos/  
Bioloogilised ohud/Risques biologiques/  
Biolóškli rizici/Biológiai kockázatok/Rischi  
biologici/Biologinis pavojus/Biológiskais  
risks/Biologische risico's/Biologische  
risikoer/Zagroženie biologiczne/Riscos  
biológicos/ Biologisk risk/Pericole  
biologice/Биологическая опасность/  
Biologicky rizikové/Biologické riziká/  
Biolóškli rizici/Biyołojik riskler



Human/C човешки произход/Lidské/  
Human/Human/ἄνθρωπος αναφοράς/  
Humano/Inimpãritolu/Humaine/Ljudskog  
porjekla/Humãn/Origine Umana/  
Žmogaus kilmės/Cilvēku izcelsmes/  
Human/Menneske/Ludzka/Humano/  
Origine umãnã/Человеческого  
происхождения/Human/Ludské/  
Humanega izvora/Ljudskog porekla/İnsan



From mouse/C миши произход/Myši/  
Fra mus/Maus/από ποντίκι/de ratón/  
Hiirtelt/De souris/Mišijeg porjekla/  
Egérbőli/Murino/Pelės kilmės/No peles/  
Van muizen/Fra mus/Mysia/Do rato/De  
la șoareci/Мышиного происхождения/  
Från mus/Myšije/Mišjega izvora/Mišijeg  
porekla/Fareden



From goat/C кози произход/ Kozli/  
Serumherkomst: Ged/Herkunft: Ziege/  
Από αίγα/De cabra/Kitsetel/ À partir de  
chèvres/Porijeklo: koza/Кецсэбőli/  
Da capra/Iš ožkos/No kazas/Van geiten/  
Fra geit/Z kozia/De cabra/De la caprã/  
козьи/Från get/Kozjega izvora/  
Od koze/Menşei: Keçi/



Reconstitute with/Разтваряне с/  
Rozfeďte pomocí/Rekonstitueres med/  
Rekonstituieren mit/Ανασύσταση με/  
Reconstituir con/Lahjendamine/  
Reconstituer avec/Rekonstituiraite s/  
Feloldáshoz/Ricostituire con/Atkurti,  
ištirpdant su/Atšķaidīt ar/Reconstituite  
met/Rekonstitueres med/Odtworzyć  
za pomocą/Reconstituir com/A  
se reconstitui cu/Разтворить в/  
Rekonstituera med/Rozriedte pomocou/  
Rekonstituiraite z/s/Ponovno formiranje  
sa/Yeniden oluşturur



Manufacturer/Производител/Výrobce/  
Producent/Hersteller/Κασκευαστής/  
Fabricante/Tootja/Fabricant/Proizvođač/  
Gyártó/Fabbricante/Gamintojas/  
Ražotājs/Fabrikant/Produsent/  
Producent/Fabricante/Producător/  
Производитель/Tilverkare/ Výrobca/  
Izdelovalec/Proizvođač/Üretici

# CanAg ProGRP EIA

Instructions for use

Enzyme immunometric assay kit

For 96 determinations

## INTENDED USE

The CanAg ProGRP EIA kit is an immunoassay for the quantitative determination of ProGRP in serum. The CanAg ProGRP assay is to be used as an aid in the differential diagnosis and in monitoring disease progression during the course of disease and treatment in small cell lung cancer patients.

Testing for patient proGRP assay values should be used in conjunction with other clinical methods used in the management of patients with small cell lung cancer.

## SUMMARY AND EXPLANATION OF THE ASSAY

GRP (Gastrin Releasing Peptide) is a hormone that 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 (1, 2). The precursor Pro Gastrin Releasing Peptide (ProGRP) however, is more stable and can be used as a serological marker for GRP (3). The CanAg ProGRPEIA kit measures ProGRP (31-98) a carboxy-terminal region common to human ProGRP splice variants.

ProGRP is expressed in neuroendocrine-derived tissues and tumors, including small cell lung cancer carcinoids, undifferentiated large cell carcinoma of the lung with neuroendocrine features, medullary thyroid carcinoma, and other neuroendocrine malignancies. Serum levels of ProGRP have been shown to be elevated in a high proportion of patients diagnosed with SCLC while normal levels are found in patients with benign disease (4-10).

Due to its high sensitivity and specificity for SCLC, ProGRP has shown clinical utility in the differential diagnosis of lung cancer. Additive information in the diagnosis of SCLC is provided by the combined measurement of ProGRP and NSE (8,10,11,14). ProGRP is also useful in monitoring the response to therapy and for the detection of recurrent disease (11-13).

Elevated levels of ProGRP may be detected in early stage small cell lung cancer (9). However, as the incidence of SCLC in the general population is low the ProGRP assay should not be used as a screening test.

## **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 patient 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 microstrips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine) 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 patient 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.
- 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
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<b>MICROPLA</b>
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<b>Microplate</b>	1 Plate	2–8°C until expiry date stated on the plate
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12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminum pouch, containing desiccant. Reseal carefully to keep dry.

<b>ProGRP Calibrators A-F</b>	6 vials, lyophilized	Stability after reconstitution 3 days at 2–8°C 3 months at -20°C or below
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<b>CAL</b>	<b>ProGRP</b>	<b>A</b>	1 x 1 mL
<b>CAL</b>	<b>ProGRP</b>	<b>B</b>	1 x 1 mL
<b>CAL</b>	<b>ProGRP</b>	<b>C</b>	1 x 1 mL
<b>CAL</b>	<b>ProGRP</b>	<b>D</b>	1 x 1 mL
<b>CAL</b>	<b>ProGRP</b>	<b>E</b>	1 x 1 mL
<b>CAL</b>	<b>ProGRP</b>	<b>F</b>	1 x 1 mL

The lyophilised calibrators contain human 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
<b>ProGRP Controls</b>	2 vials, lyophilized	Stability after reconstitution 3 days at 2–8°C 3 months at -20°C or below

CONTROL	ProGRP	1
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1 x 1 mL

CONTROL	ProGRP	2
---------	--------	---

1 x 1 mL

The lyophilized controls contain human cell-line derived ProGRP in a protein matrix and a non-azide preservative. To be reconstituted with distilled or deionised water before use. **NOTE:** The expected ProGRP concentration range is lot specific and is indicated on the label of each vial.

BIOTIN	Anti-ProGRP
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**Biotin Anti-ProGRP**

1 x 15 mL    4 months at 2–8°C

Affinity purified Biotin Anti-ProGRP Polyclonal antibody from goat, approximately 4 µg/mL. Contains phosphate buffered saline (pH 7.2), bovine serum albumin, blocking agents, detergent, an inert blue dye, and a non-azide preservative. Ready for use.

CONJ	Anti-ProGRP
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**Tracer, HRP Anti-ProGRP**

1 x 0.75 mL    4 months at 2–8°C

Stock Solution of HRP Anti-ProGRP monoclonal antibody from mouse, approximately 21 µg/mL. Contains non-azide preservatives. To be mixed with Biotin Anti-ProGRP before use.

DIL	SPE
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**Sample Diluent**

1 x 8 mL    4 months at 2–8°C

Contains 0.15 M Sodium Chloride and a non-azide preservative in a non-protein matrix. Ready for use.

Component	Quantity	Storage and stability after first use
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SUBS	TMB
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<b>TMB HRP-Substrate</b>	1 x 12 mL	2–8°C until expiry date stated on the vial
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Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetra-methylbenzidine (TMB). Ready for use.

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

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

### Indications of instability

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

### WARNINGS AND PRECAUTIONS

#### For In Vitro Diagnostic Use:

- Follow the instructions in the package insert. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Handle all patient specimens as potentially infectious. It is recommended that human source reagent and human specimens be handled in accordance with the OSHA Standard on Bloodborne pathogens (15). Biosafety level 1 (16) or other appropriate biosafety practices should be used for material that contain or are suspected of containing infectious agents.

- Avoid contact with reagents containing hydrogen peroxide or hydrochloric acid. In case of contact with any of these reagents, wash thoroughly with water.
- Follow local guidelines for disposal of all waste material.

## **SPECIMEN COLLECTION AND HANDLING**

The CanAg ProGRPEIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Plasma and other body fluids have not been validated for use with the CanAg ProGRPEIA. Serum collection tubes that contain a thrombin based clotting acceleration agent must not be used because this agent may cause degradation of ProGRP. Serum specimens should be processed immediately following adequate clotting, or stored at 2-8°C. Do not use serum specimens that have been exposed to room temperature (up to 25°C) for more than 3 hours, including clot time. Serum can be stored at 2-8°C for up to 24 hours, before being tested. Serum specimens must be tested immediately after removal from storage at 2-8°C. For longer periods store samples at -40°C or colder. Thaw frozen samples and mix thoroughly by vortexing or by inverting 10 times before analysis. For accurate results samples should be free of fibrin, red blood cells, or other particular matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.

## **PROCEDURE**

### **Materials required but not supplied with the kit**

- 1. Microplate shaker**  
Shaking should be medium to vigorous, approximately 700-1100 oscillations/min.
- 2. Microplate wash device**  
**For best results we recommend an Automatic plate washer** capable of performing 1 and 6 washing cycles, and with a minimal fill volume of **800 µL/well/wash cycle** using overflow wash mode. An 8-channel pipette with disposable plastic tips for delivery of 350 µL (i.e. completely filled wells) is recommended if an automatic microplate washer is not used.
- 3. Microplate spectrophotometer**  
With a wavelength 450 nm and an absorbance range of 0 to 3.0.
- 4. Precision pipettes**  
With disposable plastic tips for dispensing microliter 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 milliliter volumes.
- 5. Distilled or deionized water**  
For reconstitution of ProGRP Calibrators, ProGPR 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 ProGRPEIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix 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 (20–30°C) prior to use. Frozen specimens must be thoroughly mixed after thawing. **Please refer to specimen collection and handling section above.**
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. *It's highly recommended to use overflow wash mode with a dispensing volume of 800 µL.*  
**Note:** A very rapid aspiration rate in combination with no soak time may decrease the precision of the assay. 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 to 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 the well, in order to avoid carry-over. A diligent pipetting technique is of particular importance when handling the samples and the TMB HRP-Substrate solution.

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**Preparation of reagents****Stability of prepared reagent**

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**ProGRP Calibrators**

3 days at 2–8°C  
3 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 thoroughly before use.

**NOTE:** The concentration of the calibrators is stated on the labels and should be used for calculation of the results.

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**ProGRP Controls**

3 days at 2–8°C  
3 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 thoroughly before use.

**NOTE:** The expected value ranges of the controls are stated on the labels.

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**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 deionized water to give a buffered Wash Solution.

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**Antibody Solution**

12 hours 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 Protocol Sheet).

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# Protocol Sheet

**ProGRP EIA** REF **220-10**

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

Step	Vial/Plate	Procedure																																										
1. Prepare ProGRP Calibrators	<table border="1"><tr><td>CAL</td><td>ProGRP</td></tr></table> <p>A, B, C, D, E, F</p>	CAL	ProGRP	Add 1.0 mL of distilled or deionised water to each vial. Allow to stand for at least 15 minutes. Mix thoroughly before use. NOTE: The exact concentration of each calibrator is stated on the label.																																								
CAL	ProGRP																																											
Prepare ProGRP Controls	<table border="1"><tr><td>CONTROL</td><td>ProGRP</td></tr></table> <p>1, 2</p>	CONTROL	ProGRP	Reconstituted stability: 3 days at 2-8°C. 3 months at -20°C or below.																																								
CONTROL	ProGRP																																											
Prepare Wash Solution	<table border="1"><tr><td>WASHBUF</td><td>25X</td></tr></table>	WASHBUF	25X	Dilute 50 mL of Wash Concentrate with 1200 mL of distilled or deionised water.																																								
WASHBUF	25X																																											
Prepare Antibody Solution	<table border="1"><tr><td>CONJ</td><td>Anti-ProGRP</td></tr><tr><td>BIOTIN</td><td>Anti-ProGRP</td></tr></table>	CONJ	Anti-ProGRP	BIOTIN	Anti-ProGRP	Mix 50 µL of Tracer, HRP Anti-ProGRP with 1 mL of Biotin Anti-ProGRP per strip:																																						
CONJ	Anti-ProGRP																																											
BIOTIN	Anti-ProGRP																																											
	<table border="1"><thead><tr><th rowspan="2">No. of Strips</th><th rowspan="2">Tracer, HRP Anti-ProGRP (µL)</th><th colspan="2">Biotin Anti-ProGRP (mL)</th></tr><tr><th>HRP Anti-ProGRP (µL)</th><th>Anti-ProGRP (mL)</th></tr></thead><tbody><tr><td>1</td><td>50</td><td>1</td><td>1</td></tr><tr><td>2</td><td>100</td><td>2</td><td>2</td></tr><tr><td>3</td><td>150</td><td>3</td><td>3</td></tr><tr><td>4</td><td>200</td><td>4</td><td>4</td></tr><tr><td>5</td><td>250</td><td>5</td><td>5</td></tr><tr><td>6</td><td>300</td><td>6</td><td>6</td></tr><tr><td>7</td><td>350</td><td>7</td><td>7</td></tr><tr><td>8</td><td>400</td><td>8</td><td>8</td></tr><tr><td>9</td><td>450</td><td>9</td><td>9</td></tr></tbody></table>	No. of Strips	Tracer, HRP Anti-ProGRP (µL)	Biotin Anti-ProGRP (mL)		HRP Anti-ProGRP (µL)	Anti-ProGRP (mL)	1	50	1	1	2	100	2	2	3	150	3	3	4	200	4	4	5	250	5	5	6	300	6	6	7	350	7	7	8	400	8	8	9	450	9	9	
No. of Strips	Tracer, HRP Anti-ProGRP (µL)			Biotin Anti-ProGRP (mL)																																								
		HRP Anti-ProGRP (µL)	Anti-ProGRP (mL)																																									
1	50	1	1																																									
2	100	2	2																																									
3	150	3	3																																									
4	200	4	4																																									
5	250	5	5																																									
6	300	6	6																																									
7	350	7	7																																									
8	400	8	8																																									
9	450	9	9																																									



No. of Strips	Tracer, HRP Anti-ProGRP ( $\mu\text{L}$ )	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 calibrators, controls and unknown samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20-30 °C) before use.

**Please refer to specimen collection and handling section.**

1. Start to prepare ProGRP Calibrators, Controls 1 & 2, 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 aluminum 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  $\mu\text{L}$  of the ProGRP Calibrators (CAL A, B, C, D, E, and F), Controls 1 & 2 and unknown specimens (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	1st Unk				
B	Cal A	Cal E	1st Unk				
C	Cal B	Cal F	2nd Unk				
D	Cal B	Cal F	2nd Unk				
E	Cal C	C1					
F	Cal C	C1					
G	Cal D	C2					
H	Cal D	C2					

- Add 100  $\mu\text{L}$  of Antibody Solution to each well using a 100  $\mu\text{L}$  8-channel precision pipette (or a 100  $\mu\text{L}$  precision pipette). Do not allow the pipette tip to touch the surface of the liquid in order to avoid carry-over.
- Incubate the frame containing the strips for 2 hour ( $\pm 10$  min) at room temperature (20-30°C) with constant shaking of the plate using a microplate shaker.
- Wash each strip 6 times, using the wash procedure described in Procedural notes, item 4 (above).
- Add 100  $\mu\text{L}$  of TMB HRP-Substrate to each well using the same procedure as in item 4 (above). The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between addition to the first and last well should not exceed 5 min.
- Incubate for 30 min ( $\pm 5$  min) at room temperature (20-30°C) with constant shaking. Avoid exposure to direct sunlight.
- Add 100  $\mu\text{L}$  of Stop Solution to each well. Mix by using a microplate shaker and read absorbance at 450 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

## Measurement range

The CanAg ProGRP EIA calibration range is 0–2000 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, accuracy of pipettes, plate washer 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 ng/L.
- Interpolation with point-to-point evaluation. Calibrator A should be included in the curve with the value 0 ng/L.

**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.

**NOTE:** The evaluation method of calculating proGRP results should be used consistently when used in the course of monitoring a patient.

If samples in an initial analysis give ProGRP levels higher than calibrator F, then the samples should be diluted 1/10 with ProGRP Sample Diluent to obtain the accurate ProGRP concentration of the samples. Make fresh dilutions before the run. Do not exceed 1/10 dilutions.

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

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

Dilution 1/10: 10 x measured value

Samples that are above calibrator F after a dilution 1/10 is to be reported as: above 10x value of calibrator F i.e. if calibrator F is 2000 the value is reported as: above 20 000 ng/L.

## **LIMITATIONS OF THE PROCEDURE**

Patients with confirmed cancer may have ProGRP values in the same range as healthy subjects. Elevated levels of ProGRP may also be found in subjects with non-malignant disease e.g. renal failure(9). Therefore, the level of ProGRP cannot be used as absolute evidence for the presence or absence of malignant disease and the ProGRP EIA should not be used in cancer screening. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the ProGRP test should not replace any established clinical examination.

Observation pairs with both values within the normal reference range should not be used for the evaluation of disease progression.

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer. Rheumatoid factor in the patient sample may interfere with the assay causing falsely low proGRP values.

## **EXPECTED VALUES**

In a study with 626 apparently healthy individuals 99% of the subjects had ProGRP concentrations of 60 ng/L or less and 95% of the subjects had values of 43 ng/L or less. Median of serum values was 17.4 ng/L.

It is recommended that each laboratory establish its own expected reference range of interest with the population and sample collection procedures used within the laboratory.

## PERFORMANCE CHARACTERISTICS

### Precision

Total precision was determined according to NCCLS guideline EP5-A2 (17) using six levels of frozen pooled human serum containing added ProGRP. Each sample was randomly pipetted in duplicates and analysed twice each day over 20 days i.e. 40 runs with 40 different templates by two technicians repeated using 2 different ProGRP EIA kit lots. Data from this study is summarized below.

Sample	Reagent Lot	N	Mean conc. (ng/L)	Within-run SD (ng/L)	Within-run CV (%)	Total SD (ng/L)	Total CV (%)
1	1	80	33	2.3	6.9	3.0	9.2
	2	80	33	1.4	4.2	1.5	8.9
2	1	80	72	1.6	2.2	4.1	5.7
	2	80	74	1.4	1.9	2.3	6.3
3	1	80	97	3.7	3.8	7.0	7.3
	2	80	96	3.6	3.7	3.1	6.5
4	1	80	333	7.2	2.2	12.9	3.9
	2	80	326	4.3	1.3	8.3	5.1
5	1	80	781	12.8	1.6	35.6	4.6
	2	80	759	11.8	1.6	16.2	4.3
6	1	80	1449	33.8	2.3	59.2	4.1
	2	80	1495	28.8	1.9	36.4	4.9

### Detection limit

The limit of detection (LoD) corresponds to the upper limit of the 95% confidence interval and represents the lowest concentration of ProGRP antigen that can be distinguished from zero. The NCCLS guideline EP5-A2 (18) was used to design the experiments for determination of limit of detection. A study was conducted in which the ProGRP EIA Calibrator B was diluted in Calibrator A to 10 ng/L. Thereafter Calibrator A (zero) and the Calibrator B dilution (10 ng/L) were tested in replicates of 30 per run in 4 runs on two separate days. The limit of detection of the ProGRP EIA assay was found to be < 10 ng/L.

### Functional sensitivity

The functional sensitivity is expressed as the concentration of an analyte at which the total CV is 20%. A study was conducted where a five member sensitivity panel was tested in replicates of 2 in 2 runs on twenty separate days with two lots of reagents. The functional sensitivity determined for the ProGRP EIA was found to be < 20 ng/L.

## Recovery

A study was performed where dilutions of an antigen solution with known concentrations of ProGRP were added to ProGRP EIA sample diluent. The concentration of ProGRP was determined using the ProGRP EIA assay and the resulting percent recovery was calculated from the ratio of Observed ProGRP Concentration/Expected ProGRP Concentration calculated from the amount of ProGRP added. Representative data from this study is summarized in the following table\*:

Sample	ProGRP Antigen Added (ng/L)	Observed value Assay Value (ng/L)	Percent Recovery** %
1	80	94	117
	139	164	118
	742	821	111
	1464	1653	113
2	68	70	103
	128	135	106
	730	701	96
	1453	1518	104
3	79	83	105
	138	150	109
	741	790	107
	1463	1487	102
4	100	108	108
	157	178	114
	762	807	106
	1481	1590	107
5	108	116	108
	164	186	114
	770	829	108
	1489	1609	108

\*Representative data; results in individual laboratories may vary from these data.

\*\*% Recovery=Observed ProGRP Concentration (ng/L)/ProGRP added (ng/L)

The average recovery across the separate spiked concentrations shown above was found to be 108%.

## High Dose Hook

No high dose hook effect was observed for samples containing up to > 1 500 000 ng/L ProGRP antigen.

## Dilution Linearity

The ProGRP EIA assay mean dilution linearity is  $100 \pm 20\%$ . A study was conducted for the ProGRP EIA modeled after the NCCLS (CLSI) guideline EP6-A (19). Serum samples with elevated ProGRP values were diluted with ProGRP EIA Sample Diluent. The ProGRP concentration was determined for each dilution and the percent (%) recovery was calculated. Data from a representative sample from this study is presented in the following table\*:

Sample	Final Dilution Factor	Obtained Value (ng/L)	Expected Value (ng/L)	Percent Recovery** (%)
A	Undiluted	1526	1526	100
	1:1.11	1344	1373	98
	1:1.25	1242	1221	102
	1:1.67	901	915	98
	1:2.5	636	610	104
	1:5	342	304	112
	1:10	147	152	97

Data from a a linearity study using the recommended 1:10 dilutions is presented in the following table\*:

Sample	Final Dilution Factor	Obtained Value (ng/L)	Expected Value (ng/L)	Percent Recovery** (%)
A	Undiluted	1025	1025	100
	1:10	103	103	101
B	Undiluted	434	434	100
	1:10	48	43	111
C	Undiluted	1156	1156	100
	1:10	115	115	99

\*Representative data; results in individual laboratories may vary from these data.

\*\*% Recovery= ProGRP Concentration obtained x Dilution factor/Undiluted ProGRP Concentration.

Average recovery across the three diluted samples was found to be 103.6%.

## Analytical Specificity

The ProGRP EIA assay mean assay specificity is  $100\pm 15\%$ . Recovery studies were performed to compare sera containing the following compounds at the indicated concentrations with control sera. The NCCLS guideline EP7-A (20) was used to design the interference experiments. The following substances and concentrations were tested and found not to interfere with the test.

Endogenous serum interferences	Test Concentration
Triglycerides	30 mg/mL
Billirubin	0.2 mg/mL
Hemoglobin	4.5 mg/mL*
Total Protein	120 mg/mL

\* For serum samples with hemoglobin concentrations above 4.5 mg/mL there is a risk of getting an elevated result with the ProGRP EIA.

Chemotherapeutic drug interferences	Test Concentration
Carboplatin	500 µg/mL
Cisplatin	165 µg/mL
Dexamethasone	10 µg/mL
Doxorubicin	1.16 µg/mL
Leucovorin	2.68 µg/mL
Methotrexate	45 µg/mL
Paclitaxel	3.5 ng/mL

## Potentially interfering clinical conditions

The ProGRP EIA assay was evaluated using specimens with HAMA and Rheumatoid Factor (RF) to further assess the assay specificity. Six specimens positive for HAMA and five specimens positive for RF were evaluated for percent recovery with ProGRP antigen spiked into each specimen at low and high concentrations.

**HAMA:** The grand mean recovery of ProGRP in the presence of HAMA was 101% and the individual recoveries ranged from 93–111%.

**RF:** The grand mean recovery was 79%, in specimens positive for Rheumatoid factor. Patients with abnormal levels of rheumatoid factor may thus have underestimated ProGRP values.

## WARRANTY

Any change or modification of the procedure not recommended by Fujirebio Diagnostics may affect the results, in which event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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