

DRG[®] Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)



Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

This Human GIP (Total) ELISA kit is used for the non-radioactive quantification of Human GIP in human serum, plasma, tissue extract and cell culture samples.

This kit has 100% cross reactivity to human GIP(1-42) and GIP(3-42).

One kit is sufficient to measure 39 unknown samples in duplicate.

This kit is for research purpose only.

2 PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of human GIP molecules from samples to the wells of a microtiter plate coated by a pre-titrated amount of anti-GIP monoclonal antibodies, 2) wash away of unbound materials from samples, 3) binding of a second biotinylated anti-GIP polyclonal antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) incubation of streptavidin-Horseradish peroxidase conjugate to bind to the immobilized biotinylated antibodies, 6) wash away of free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured human GIP in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human GIP.

3 REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

A. GIP ELISA Plate

Coated with anti-GIP Monoclonal Antibodies

Quantity: 1 plate

Preparation: Ready to Use

Note: Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C.

B. Adhesive Plate Sealer

Quantity: 2 sheets

Preparation: Ready to Use

C. 10X HRP Wash Buffer Concentrate

10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.

Quantity: 2 bottles containing 50 ml each

Preparation: Dilute 1:10 with distilled or deionized water.

DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)



D. Human GIP Standard

Human GIP(1-42), 0.5 ml/vial, lyophilized.

Quantity: 0.5 ml/vial upon hydration

Preparation: Reconstitute with 0.5 ml distilled or deionized water.

The actual concentration of Human GIP present in the vial will be lot dependent. Please refer to the analysis sheet for exact Human GIP concentration present in a specific lot.

E. Human GIP Quality Controls 1 and 2

Human GIP(1-42), 0.5 ml/vial, lyophilized.

Quantity: 0.5 ml/vial upon hydration

Preparation: Contents lyophilized. Reconstitute with 0.5 ml distilled or deionized water.

F. Assay Buffer

Buffer containing BSA and 0.08% Sodium Azide

Quantity: 12 ml

Preparation: Ready to Use

G. Human GIP Detection Antibody

Pre-titered Biotinylated Rabbit anti-Human GIP Polyclonal Antibody

Quantity: 11 ml

Preparation: Ready to Use

H. Enzyme Solution

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer

Quantity: 12 ml

Preparation: Ready to Use

I. Substrate (*Light sensitive, avoid unnecessary exposure to light*)

3, 3', 5, 5'-tetramethylbenzidine in buffer

Quantity: 12 ml

Preparation: Ready to Use.

J. Stop Solution (*Caution: Corrosive Solution*)

0.3 M HCl

Quantity: 12 ml

Preparation: Ready to Use

K. Matrix Solution

Quantity: 1 ml/vial

Preparation: Ready to Use

DRG[®] Human GIP ELISA (EIA-4527)**REVISED 23 FEB. 2010 RM (VERS. 3.0)****4 STORAGE AND STABILITY**

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C.

For longer storage (> 2 weeks), freeze Wash Buffer, Assay Buffer, Matrix Solution, and reconstituted Standards and Controls at ≤ -20°C.

Minimize repeated freeze and thaw of the GIP Standards and Quality Controls.

Unused microtiter strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C.

Refer to expiration dates on all reagents prior to use.

Do not mix reagents from different kits unless they have the same lot numbers.

5 REAGENT PRECAUTIONS**A. Sodium Azide**

Sodium azide has been added to certain reagents as a preservative. Although the concentrations are low, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

B. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10 µL - 20 µL or 20 µL - 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 5 ~ 50 µL and 50 ~ 300 µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

7 SAMPLE COLLECTION AND STORAGE

NOTE: Although DPP IV inhibitor is not required to be added to serum/plasma samples for measurement of total GIP, we recommend that DPP IV inhibitor be added to the serum/plasma samples during the sample collection so that the same samples could be used in the future for the measurement of intact (1-42) GIP with an assay that is capable of selectively measuring only the intact GIP.

DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)

RUO

1. To prepare **serum samples**, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.

Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at $4 \pm 2^{\circ}\text{C}$.

Transfer and store serum samples in separate tubes. Date and identify each sample.

Use freshly prepared serum or aliquot and store samples at $\leq -20^{\circ}\text{C}$ for later use. For long-term storage, keep at -70°C . Avoid freeze/thaw cycles.

2. To prepare **plasma samples**, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/ml and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.
3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
4. Avoid using samples with gross hemolysis or lipemia.

8 SAMPLE PREPARATION

1. No dilution or preparation is needed for normal serum or plasma samples. In the event that any sample is above 2000 pg/mL range, dilutions should be performed using the Matrix Solution provided.
2. Tissue extracts or cell culture media samples may require dilution. Dilutions should be performed using the assay buffer provided.

9 STANDARD AND QUALITY CONTROLS PREPARATION

9.1 Human GIP Standard Preparation

1. Use care in opening the lyophilized Standard vial.
Using a pipette, reconstitute the Human GIP Standard with 0.5 ml distilled or deionized water into the glass vial to give a concentration prescribed in the analysis sheet. .
Invert and mix gently, and let sit for 5 minutes then mix well.
2. Label five tubes 1, 2, 3, 4 and 5. Add 100 μL Assay Buffer to each of the five tubes.
Perform 3 times serial dilutions by adding 50 μL of the reconstituted standard to Tube 1,
mix well and transfer 50 μL of Tube 1 to Tube 2,
mix well and transfer 50 μL of Tube 2 to Tube 3,
mix well and transfer 50 μL of Tube 3 to Tube 4,
mix well and transfer 50 μL of Tube 4 to Tube 5 and mix well.

Note:

Do not use a repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at $\leq -20^{\circ}\text{C}$. Avoid multiple freeze/thaw cycles.

DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)

RUO

| Standard Concentration pg/mL | Volume of Deionized Water to Add | Volume of Standard to Add |
|---|-------------------------------------|------------------------------|
| X (refer to the analysis sheet for exact concentration) | 0.5 ml | 0 |

| Tube # | Standard Concentration pg/mL | Volume of Assay Buffer to Add | Volume of Standard to Add |
|--------|---------------------------------|----------------------------------|------------------------------------|
| 1 | X/3 | 100 µL | 50 µL of Reconstituted Standard |
| 2 | X/9 | 100 µL | 50 µL of Tube 1 |
| 3 | X/27 | 100 µL | 50 µL of Tube 2 |
| 4 | X/81 | 100 µL | 50 µL of Tube 3 |
| 5 | X/243 | 100 µL | 50 µL of Tube 4 |

9.2 Human GIP Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials.

Using a pipette, reconstitute each of the Human GIP Quality Control 1 and Quality Control 2 with 0.5 ml distilled or deionized water into the glass vials.

Invert and mix gently, let sit for 5 minutes then mix well.

10 ASSAY PROCEDURE

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 ml deionized or distilled water.
2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C.
Assemble strips in an empty plate holder and **fill each well with 300 µL of diluted Wash Buffer**. Incubate at room temperature **for 5 minutes**. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol.**
3. Add in duplicate, **80 µL Assay Buffer** to the **blank** wells and **sample** wells.
(See plate well map for suggested well orientation).
4. Add in duplicate, **60 µL Assay Buffer** to **Standard** wells, **QC1** and **QC2** wells.
5. Add **20 µL Matrix Solution** to the **Blank** wells, **Standard** wells, and **QC1** and **QC2** wells.

DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)

RUO

6. Add in duplicate, **20 µL Human GIP Standards** in the order of ascending concentration to the appropriate wells. Add in duplicate, **20 µL QC1 and 20 µL QC2** to the appropriate wells. Add sequentially, **20 µL of the unknown samples** in duplicate to the remaining wells.
For best result all additions should be completed within 30 minutes.
7. Cover the plate with plate sealer and incubate at **room temperature for 1.5 hours** on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
8. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
9. **Wash wells 3 times** with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
10. Add **100 µL Detection Antibody to all wells**. Cover the plate with plate sealer and incubate at **room temperature for 1 hour** on an orbital microtiter **plate shaker** set to rotate at moderate speed, approximately 400 to 500 rpm.
11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
12. **Wash wells 3 times** with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
13. Add **100 µL Enzyme Solution** to each well. Cover plate with sealer and incubate with moderate shaking at **room temperature for 30 minutes** on the microtiter **plate shaker**.
14. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
15. **Wash wells 3 times** with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
16. Add **100 µL of Substrate Solution** to each well, cover plate with sealer and shake on the plate shaker for **approximately 8 to 20 minutes** (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of GIP standards with intensity proportional to increasing concentrations of GIP.
NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time. One can monitor color development using 370 nm filter, if available on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.
17. Remove sealer and add **100 µL Stop Solution** [CAUTION: CORROSIVE SOLUTION] and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. **Read absorbance** at 450 nm and 590nm in a plate reader **within 5 minutes** and ensure that there are no air bubbles in any well.
Record the difference of absorbance units. The absorbance of the highest GIP standard should be approximately 2.0-3.2, or not to exceed the capability of the plate reader used.

DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)

RUO

11 MICROTITER PLATE ARRANGEMENT

Human GIP (Total) ELISA

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|----------|--------|---------------|--------|---|---|---|---|---|---|----|----|----|
| A | Blank | Tube 2 | QC 2 | | | | | | | | | |
| B | Blank | Tube 2 | QC 2 | | | | | | | | | |
| C | Tube 5 | Tube 1 | Sample | | | | | | | | | |
| D | Tube 5 | Tube 1 | Sample | | | | | | | | | |
| E | Tube 4 | Reconst . Std | Sample | | | | | | | | | |
| F | Tube 4 | Reconst . Std | Sample | | | | | | | | | |
| G | Tube 3 | QC 1 | Sample | | | | | | | | | |
| H | Tube 3 | QC 1 | Etc. | | | | | | | | | |

12 CALCULATIONS

The dose-response curve of this assay fits best to a 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function.

Note:

When sample volumes assayed differ from 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µL, compensate the volume deficit with matrix solution.

13 INTERPRETATION

Acceptance Criteria

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.
3. The limit of sensitivity of this assay is 8.2 pg/mL human GIP (20 µL sample size).

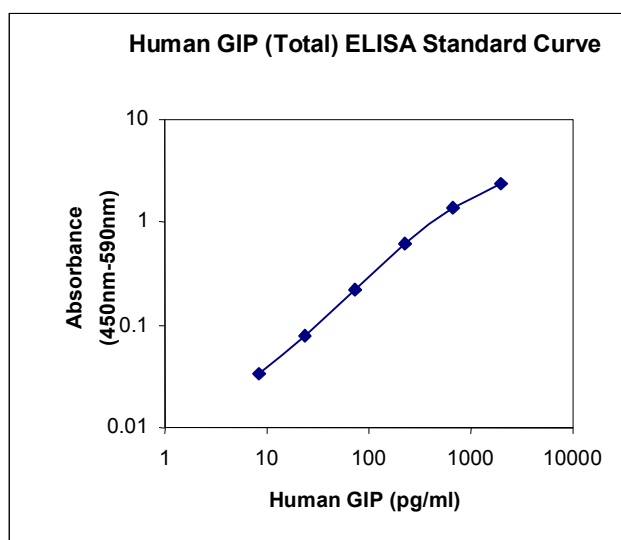
DRG[®] Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)

RUO

4. The appropriate range of this assay is 8.2 pg/mL to 2000 pg/mL human GIP (20 μ L sample size). Any result greater than 2000 pg/mL in a 20 μ L sample should be diluted using matrix solution, and the assay repeated until the results fall within range.
Tissue extracts or cell culture media samples greater than 2000 pg/mL in a 20 μ L sample should be diluted in Assay Buffer.

14 STANDARD CURVE



Typical Standard Curve, not to be used to calculate results.

15 ASSAY CHARACTERISTICS

15.1 Sensitivity

The lowest level of Human GIP that can be detected by this assay is 8.2 pg/mL when using a 20 μ L sample size.

15.2 Specificity

The antibody pair used in this assay is specific to human GIP and does not significantly cross-react with Glucagon, Oxyntomodulin, GLP-1 and GLP-2.

DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)

RUO

15.3 Precision

Intra-Assay Variation

| Sample No. | Mean GIP Levels (pg/mL) | Intra-Assay %CV |
|------------|----------------------------|--------------------|
| 1 | 15 | 6.7 |
| 2 | 21 | 7.3 |
| 3 | 279 | 3.0 |
| 4 | 185 | 8.8 |

Inter-Assay Variation

| Sample No. | Mean GIP Levels (pg/mL) | Inter-Assay %CV |
|------------|----------------------------|--------------------|
| 1 | 26 | 6.1 |
| 2 | 50 | 3.3 |
| 3 | 134 | 2.3 |
| 4 | 166 | 1.8 |

The assay variations of GIP (Total) ELISA kits were studied on 8 human serum samples with varying concentrations of endogenous GIP.

The mean intra-assay variation was calculated from results of six duplicate determinations in each assay of the indicated samples.

The mean inter-assay variations of each sample were calculated from results of four separate assays with duplicate samples in each assay.

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RUO

15.4 Recovery

Spike & Recovery of Human GIP in Human Serum Samples

| Sample No. | GIP Added (pg/mL) | Expected (pg/mL) | Observed (pg/mL) | % of Recovery |
|------------|-------------------|------------------|------------------|---------------|
| 1 | 0 | 76 | 76 | 100 |
| | 49.4 | 125.4 | 111 | 89 |
| | 148.9 | 224.9 | 200 | 89 |
| | 444.4 | 520.4 | 481 | 90 |
| 2 | 0 | 58 | 58 | 100 |
| | 49.4 | 107.4 | 93 | 87 |
| | 148.9 | 206.9 | 167 | 81 |
| | 444.4 | 502.4 | 418 | 83 |
| 3 | 0 | 54 | 54 | 100 |
| | 49.4 | 103.4 | 89 | 86 |
| | 148.9 | 202.9 | 176 | 87 |
| | 444.4 | 498.4 | 441 | 88 |

Varying amounts of human GIP were added to three human serum samples and the GIP content was determined in three separate assays.

The % of recovery = observed GIP concentrations/expected GIP concentrations x 100%.

15.5 Linearity

Effect of Serum Dilution

| Sample No. | Volume Sampled (μL) | Expected (pg/mL) | Observed (pg/mL) | % Of Expected |
|------------|---------------------|------------------|------------------|---------------|
| 1 | 20 | 396 | 396 | 100 |
| | 10 | 198 | 200 | 101 |
| | 5 | 99 | 102 | 103 |
| | 2.5 | 49.5 | 50 | 101 |
| 2 | 20 | 363 | 363 | 100 |
| | 10 | 181.5 | 176 | 97 |
| | 5 | 90.75 | 89 | 98 |
| | 2.5 | 45.4 | 45 | 99 |
| 3 | 20 | 648 | 648 | 100 |
| | 10 | 324 | 310 | 96 |
| | 5 | 162 | 162 | 100 |
| | 2.5 | 81 | 84 | 104 |

DRG[®] Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)



Three human serum samples with the indicated sample volumes were assayed. Required amounts of matrix were added to compensate for lost volumes below 20 µL.

The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 representing 20 µL, 10 µL, 5 µL, and 2.5µl sample volumes assayed, respectively, were applied in the calculation of observed GIP concentrations.

% expected = observed/expected x 100%.

16 QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert.

17 TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to
 - 1) cross well contamination by standard solution or sample or
 - 2) inadequate washing of wells with Wash Buffer or
 - 3) overexposure to light after substrate has been added.

18 ORDERING INFORMATION

Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals. All products are intended for in vitro use only.

Material Safety Data Sheets (MSDS)











Material safety data sheets for DRG products may be ordered by fax or phone.








DRG® Human GIP ELISA (EIA-4527)

REVISED 23 FEB. 2010 RM (VERS. 3.0)



SYMBOLS USED WITH DRG ASSAYS

| Symbol | English | Deutsch | Français | Español | Italiano |
|---|------------------------------------|------------------------------|--|---------------------------------------|-------------------------------------|
|  | Consult instructions for use | Gebrauchsanweisung beachten | Consulter les instructions d'utilisation | Consulte las instrucciones de uso | Consultare le istruzioni per l'uso |
|  | European Conformity | CE-Konformitätskennzeichnung | Conformité aux normes européennes | Conformidad europea | Conformità europea |
|  | In vitro diagnostic device | In-vitro-Diagnostikum | Usage Diagnostic in vitro | Para uso Diagnóstico in vitro | Per uso Diagnostica in vitro |
|  | For research use only | Nur für Forschungszwecke | Seulement dans le cadre de recherches | Sólo para uso en investigación | Solo a scopo di ricerca |
|  | Catalogue number | Katalog-Nr. | Numéro de catalogue | Número de catálogo | Numero di Catalogo |
|  | Lot. No. / Batch code | Chargen-Nr. | Numéro de lot | Número de lote | Numero di lotto |
|  | Contains sufficient for <n> tests/ | Ausreichend für "n" Ansätze | Contenu suffisant pour "n" tests | Contenido suficiente para <n> ensayos | Contenuto sufficiente per "n" saggi |
|  | Storage Temperature | Lagerungstemperatur | Température de conservation | Temperatura de conservación | Temperatura di conservazione |
|  | Expiration Date | Mindesthaltbarkeits-datum | Date limite d'utilisation | Fecha de caducidad | Data di scadenza |
|  | Legal Manufacturer | Hersteller | Fabricant | Fabricante | Fabbricante |
| Distributed by | Distributor | Vertreiber | Distributeur | Distribuidor | Distributore |
| Content | Content | Inhalt | Conditionnement | Contenido | Contenuto |
| Volume/No. | Volume / No. | Volumen/Anzahl | Volume/Quantité | Volumen/Número | Volume/Quantità |

| Symbol | Portugues | Dansk | Svenska | Ελληνικά |
|---|--------------------------------------|--|---|---------------------------------------|
|  | Consulte as instruções de utilização | Se brugsanvisning | Se bruksanvisningen | Εγχειρίδιο χρήστη |
|  | Conformidade com as normas europeias | Europaeisk overensstemmelse | Europeisk överensstämmelse | Ευρωπαϊκή Συμμόρφωση |
|  | Diagnóstico in vitro | In vitro diagnostik | Diagnostik in vitro | in vitro διαγνωστικό |
|  | | | | |
|  | Catálogo n.º | Katalognummer | Katalog nummer | Αριθμός καταλόγου |
|  | No do lote | Lot nummer | Batch-nummer | Αριθμός Παρτίδος |
|  | | Indeholder tilstrækkeligt til "n" test | Innehåller tillräckligt till "n" tester | Περιεχόμενο επαρκές για «n» εξετάσεις |
|  | Temperatura de conservação | Opbevarings-temperatur | Förvaringstemperatur | Θερμοκρασία αποθήκευσης |
|  | Prazo de validade | Udløbsdato | Bäst före datum | Ημερομηνία λήξης |
|  | Fabricante | Producent | Tillverkare | Κατασκευαστής |
| Content | Conteúdo | Indhold | Innehåll | Περιεχόμενο |
| Volume/No. | Volume/Número | Volumen/antal | Volym/antal | Όγκος/αριθ.. |