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Not for use in diagnostic procedures.

hGH ELISA

Version: 09
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Colorimetric enzyme immunoassay for the quantitative determination of secreted human growth hormone (hGH) from *E. coli* in transfected eukaryotic cells

Cat. No. 11 585 878 001 1 kit
192 tests

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	blue	hGH ELISA, hGH human growth hormone	▪ Recombinant protein (22 kDa) from *E. coli.* ▪ Lyophilized, stabilized ▪ Purity >98% (SDS PAGE) ⚠ ***See lot-specific label for exact content in nanograms.***	1 bottle, approximately 5 ng
2	white	hGH ELISA, Anti-hGH-DIG	▪ Polyclonal antibody to hGH (from sheep), conjugated to digoxigenin. ▪ Lyophilized, stabilized	1 bottle, 50 µg
3	red	hGH ELISA, Anti-DIG-POD (HRP)	▪ Polyclonal antibody to digoxigenin (from sheep), conjugated to peroxidase. ▪ Lyophilized, stabilized	1 bottle, 10 U
4	green	hGH ELISA, POD substrate, ABTS	▪ ABTS substrate solution. ▪ Ready-to-use solution, stabilized.	1 bottle, 100 ml
5	green	hGH ELISA, Substrate enhancer	Powder ⚠ ***Use Substrate enhancer only if the hGH concentration is low.***	1 bottle, 150 mg
6	colorless	hGH ELISA, Washing buffer, 10x conc.	PBS (phosphate-buffered saline), containing Tween 20, 10x conc. solution.	1 bottle, 120 ml
7	red	hGH ELISA, Sample buffer	▪ Contains PBS, containing blocking reagents. ▪ Ready-to-use solution.	2 bottles, 100 ml each
8	foil bag	hGH ELISA, Microplate	▪ Pre-coated with a polyclonal antibody to hGH (from sheep) and post-coated with blocking reagent. ▪ Shrink-wrapped with a desiccant capsule (24 × 8 wells).	2 strip frames, 12 modules of 8 wells each
9	-	hGH ELISA, Self-adhesive Plate Cover Foil	Prevents evaporation. ⚠ ***Cover the Microplate modules with the Cover Foils during each incubation step.***	6 foils

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	hGH human growth hormone	Store at +2 to +8°C.
2	white	Anti-hGH-DIG	
3	red	Anti-DIG-POD (HRP)	
4	green	POD substrate, ABTS	
5	green	Substrate enhancer	
6	colorless	Washing buffer, 10x conc.	
7	red	Sample buffer	
8	foil bag	Microplate	
9	–	Self-adhesive Plate Cover Foil	

1.3. Additional Equipment and Reagent required

For the Preparation of Kit Working Solutions

- Double-distilled water

1.4. Application

The hGH ELISA is used to quantitatively measure hGH expression released into the cell culture supernatant of eukaryotic cells transfected with a plasmid bearing a hGH-encoding reporter gene.

1.5. Preparation Time

Assay Time

Approximately 4 hours.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The hGH ELISA is used with supernatants from transfected cells, no less than approximately 18 hours post transfection.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Prepare the working solutions according to the following table.

⚠ ***To avoid confusion, label each solution with the appropriate solution number (solutions 1 to 7).***

ⓘ *Always use double-distilled water for reconstitution and dilution of reagents.*

Solution	Content	Reconstitution/ Preparation of Working Solution	Storage and Stability	For use in...
1	hGH human growth hormone stock solution (Bottle 1)	▪ Reconstitute the lyophilizate in 0.5 ml double-distilled water. ▪ The resulting concentration is calculated using the lot-specific information on the bottle (final concentration 10 ng/ml).	Store 2 months at +2 to +8°C, or 12 months at or below −15 to −25°C in aliquots.	▪ Preparation of hGH human growth hormone working solution. ▪ Preparation of hGH Standards to prepare an hGH calibration curve (see, **Preparation of hGH Standards**).
2	Anti-hGH-DIG (Bottle 2)	Reconstitute the lyophilizate in 0.5 ml double-distilled water (final concentration 100 µg/ml).	Store 2 months at +2 to +8°C, or 12 months at or below −15 to −25°C in aliquots.	For solution 2a.
2a	Anti-hGH-DIG, working dilution	Dilute the reconstituted anti-hGH-DIG solution (100 µg/ml) with Sample buffer (solution 7) to a final concentration of 1 µg/ml (e.g., 100 µl of reconstituted anti-hGH-DIG solution plus 9.9 ml of solution 7 for 50 wells).	Always prepare fresh before use; do not store.	ELISA Assay
3	Anti-DIG-POD (Bottle 3)	Reconstitute the lyophilizate in 0.5 ml double-distilled water (final concentration 20 U/ml). ⚠ ***Do not add sodium azide; it inhibits the activity of the peroxidase.***	Store 6 months at +2 to +8°C. ⚠ ***Do not freeze.***	For solution 3a.
3a	Anti-DIG-POD, working dilution	Dilute the reconstituted anti-DIG-POD solution (20 U/ml) with Sample buffer (solution 7) to a final concentration of 200 mU/ml (e.g., 100 µl of reconstituted anti-DIG-POD solution plus 9.9 ml of Sample buffer for 50 wells).	Always prepare fresh before use; do not store.	ELISA Assay
4	POD substrate (Bottle 4)	Ready-to-use ABTS solution.	Store at +2 to +8°C until the kit expiration date.	ELISA Assay, for solution 5 (optional)

5	ABTS substrate solution containing Substrate enhancer (Bottles 4 and 5)	Add 1 mg of Substrate enhancer (Bottle 5) per ml of ABTS substrate solution (solution 4) and mix by stirring for 30 minutes at +15 to +25°C. *Use the Substrate enhancer only if the hGH concentration is low.*	Stable only 2 hours; prepare immediately before use.	ELISA Assay (optional)
6	Washing buffer, 1x (Bottle 6)	To prepare a ready-to-use Washing buffer, mix 1 part of the Washing buffer, 10x concentrated (Bottle 6) with 9 parts of double-distilled water. *The total amount of Washing buffer, 1x required for all washing steps is 3 ml (3 × 1 ml) per well.*	Store 6 months at +2 to +8°C.	ELISA Assay
7	Sample buffer (Bottle 7)	Ready-to-use solution. *Mix thoroughly before use.* ***Do not add sodium azide.***	Store the solution in aliquots at −15 to −25°C since it does not contain any preservatives.	▪ Preparation of Anti-DIG-POD and Anti-hGH-DIG working dilutions. ▪ For preparation of hGH working dilution, see **Preparation of hGH Standards**. ▪ For dilution of samples.
8	Microplate	Use only the Microplate modules required for the particular experiment. Close the foil bag containing the remaining modules and the desiccant capsule tightly with adhesive tape. *The anti-hGH-coated Microplate modules are ready-to-use and do not need to be rehydrated before use.*	Once, the foil bag is opened, store Microplate modules desiccated at +2 to +8°C for a minimum of 2 weeks.	ELISA Assay

2.2. Protocols

Sample Preparation

① Use cell culture supernatants directly as sample material in the hGH ELISA, without centrifugation.

② When testing culture supernatants with high hGH concentrations, dilute the medium with sample buffer (solution 7) or PBS and add 200 µl of these dilutions to the wells.

③ hGH is stable in culture medium for several months at −15 to −25°C if stored without repeated freezing and thawing, or for several days at +2 to +8°C.

Measurement of hGH

The amount of sample material required in the assay depends on the level of expression. The type of promoter, type of expression (stable versus transient), and cell type all affect the amount of hGH produced. In general, it is recommended to start with 50 µg or 1×10^3 cells. Most of the available ELISA plate readers reach their absorbance maximum at approximately 2 to 2.5 absorbance units. Measurement of supernatants with high hGH concentrations therefore requires dilution of the supernatants with Sample buffer (solution 7).

Substrate Enhancer

The use of the ABTS substrate solution containing substrate enhancer (solution 5) approximately doubles the sensitivity of the assay.

1. Remove the POD substrate ABTS without substrate enhancer (solution 4), when the test (see section, **ELISA Assay**) is first performed under sub-optimal conditions.
2. Wash each well two times with washing buffer (solution 6).
3. Incubate with POD substrate ABTS with substrate enhancer (solution 5).
4. Using the same protocol, an incubation with substrate buffer with substrate enhancer may be followed by an incubation with POD substrate solution without substrate enhancer to improve detection range.

Non-Linear Calibration Curves

Prolonged incubation of the samples with the ABTS peroxidase substrate, such as overnight at +2 to +8°C, can produce a non-linear calibration curve and is therefore only recommended for qualitative analysis of hGH expression. Since the POD substrates (solutions 4 and 5) are slightly colored, leave one well empty in order to determine the blank (baseline) value. Add POD substrate to this well for use as a reference when measuring the Microplate modules in the ELISA reader. Most readers can be programmed to automatically subtract the reference (blank) value from the values of the other samples.

Preparation of hGH Standards for Calibration Curve

Add 40 µl hGH stock solution (approximately 10 ng/ml, solution 1) to 960 µl Sample buffer (solution 7, final concentration approximately 400 pg/ml; depending on the lot-specific content).
Follow these guidelines when handling the hGH Standards.

① Prepare hGH standard dilutions directly before use. Do not store.

② Prepare the standard dilution series in reaction tubes in 1:2 dilution steps as described in the table below.

③ To obtain a calibration curve, use the 7 concentrations shown.

④ 200 µl of each dilution is needed per well.

⑤ To ensure that the measurements and the calibration curve are accurate, prepare two samples of each concentration for measurement.

⑥ To avoid carryover of the higher-concentrated solution to the lower-concentrated samples, use a fresh pipette tip for each dilution step.

⑦ Measure each dilution in duplicate.

Step	hGH Working Dilution [approximately 400 pg/ml]	Add Sample Buffer (solution 7) [µl]	Approximate hGH Concentration [pg/ml]
0	0	1,000	0
1	1,000 µl	0	400
2	500 µl Step 1	500	200
3	500 µl Step 2	500	100
4	500 µl Step 3	500	50
5	500 µl Step 4	500	25
6	500 µl Step 5	500	12.5

Microplate Pipetting Scheme

Set up the pipetting scheme according to the following table.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B1	B1	P1	P1	–	–	–	–	–	–	–	–
B	S1	S1	P2	P2	–	–	–	–	–	–	–	–
C	S2	S2	–	–	–	–	–	–	–	–	–	–
D	S3	S3	–	–	–	–	–	–	–	–	–	–
E	S4	S4	–	–	–	–	–	–	–	–	–	–
F	S5	S5	–	–	–	–	–	–	–	–	–	–
G	S6	S6	–	–	–	–	–	–	–	–	–	–
H	S7	S7	–	–	–	–	–	–	–	–	P32	P32

B1 = blank (= POD substrate)
S1 to S7 = hGH standard dilutions
P1 to P32 = samples 1 to 32

ELISA Assay

Use only the Microplate modules required for the particular experiment and place them in the frame in the correct orientation. Correct fitting ensures a tight support of the Microplate modules. The modules are ready-to-use and do not need to be rehydrated prior to addition of the samples.

⚠ ***Equilibrate reagents to +15 to + 25°C before starting the assay. Reagents from kits with different lot numbers must not be used in one assay series.***

1. Pipette 200 µl of hGH standard working dilutions (see section, **Preparation of hGH Standards)** or 200 µl culture supernatants per well.
   – Cover the Microplate modules with a cover foil and incubate for 1 hour at +37°C.

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2. Remove the solution.
   – Rinse wells 5 times with 250 µl of washing buffer (solution 6) for 30 seconds each.
   – Carefully remove washing buffer.

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3. Pipette 200 µl of anti-hGH-DIG working dilution (solution 2a) per well.
   – Cover the Microplate modules with the cover foil and incubate for 1 hour at +37°C.

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4. Remove the solution.
   – Rinse wells 5 times with 250 µl of washing buffer (solution 6) for 30 seconds each.
   – Carefully remove washing buffer.

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5. Pipette 200 µl of anti-DIG-POD working dilution (solution 3a) per well.
   – Cover the Microplate modules with the cover foil and incubate for 1 hour at +37°C.

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6. Remove the solution.
   – Rinse wells 5 times with 250 µl of washing buffer (solution 6) for 30 seconds each.
   – Carefully remove washing buffer.

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7. Pipette 200 µl of POD substrate without (solution 4) or POD substrate with substrate enhancer (solution 5) into each well.
   i *Use the substrate enhancer only if the hGH concentration is low.*
   – Incubate at +15 to +25°C until color development (green color) is sufficient for photometric detection (10 to 30 minutes).
   – Shake Microplates at 250 rpm during incubation with substrate solution to shorten the incubation period (optional). If shaking is not carried out, gently tap on the side of the Microplate before measuring absorbance to ensure a homogeneous distribution of the colored reaction product.

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8. Measure the absorbance of the samples at 405 nm (reference wavelength: approximately 490 nm) using a microplate (ELISA) reader, such as the EAR 340 ATTC, SLT Lab Instruments.

2.3. Parameters

Cross Reactivity

No cross-reaction with rat growth hormone. Cross-reactivity with the human homologues TSH, FSH, and LH has not been tested.

Purity

hGH Standard: >98% (SDS PAGE)
The hGH protein from *E. coli*, included in the kit for the purpose of compiling a standard calibration curve, is provided with lot-specific content data as determined by immunoassay.

Sensitivity

≥5 pg/ml (≥1 pg/well)

Specificity

The hGH ELISA specifically detects human growth hormone (hGH).

3. Results

Interpretation of Results

After completing the protocol, calculate the exact hGH concentration (pg/ml) of the calibration standards. Plot the absorbance values obtained on the Y-axis against the hGH standard concentrations on the X-axis to obtain a calibration curve (Fig. 2).

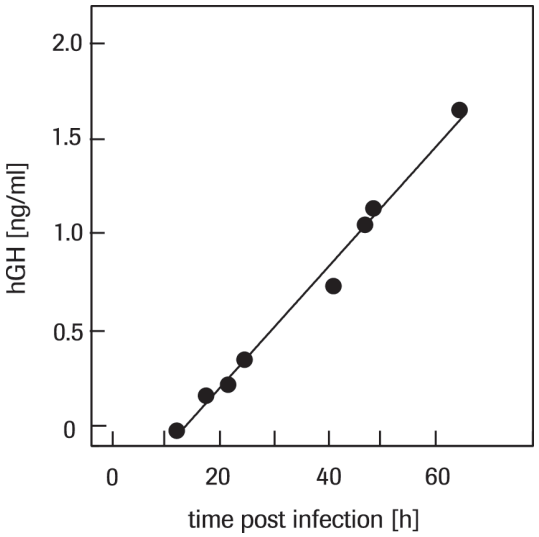

Fig. 1: HeLa cells were grown in culture medium supplemented with 10% fetal calf serum (FCS). Cells (0.8 × 106) were seeded in 6 cm dishes for 14 hours before transfection. Cells were transfected with 5 µg of the hGH-expressing vector pXGH5 using the transfection reagent DOTAP* (6 µg DOTAP per 1 µg DNA) in medium, supplemented with 10% FCS. The release of hGH into the culture supernatant was determined using the hGH ELISA. To achieve measurement of hGH in the linear range of the assay, aliquots of the culture supernatants were diluted with sample buffer (solution 7). The amount of hGH in the supernatant was determined by comparing absorbance values with those of a calibration curve. hGH concentrations of unknown samples are obtained by plotting the observed absorbance values on the Y-axis, extrapolating to meet the calibration curve, and reading the resulting hGH concentration from the X-axis. To obtain reliable results, the absorbance values of the sample should be within the linear portion of the calibration curve.

To allow direct, quantitative comparison of data obtained in independent experiments, a separate calibration curve must be established for each series.

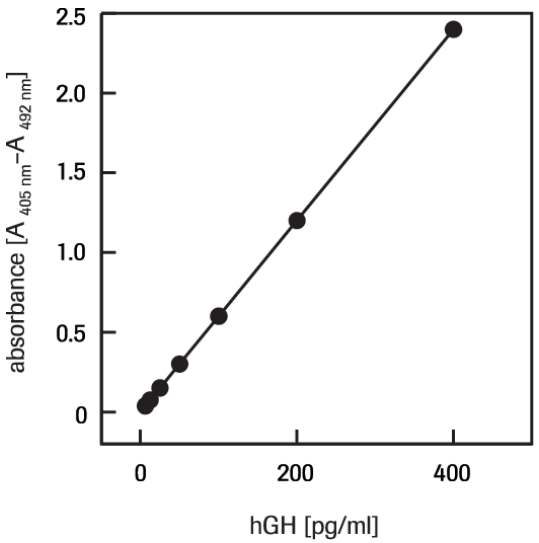

Fig. 2: A typical calibration curve using the POD substrate, ABTS after a 15 minute color reaction.

4. Additional Information on this Product

4.1. Test Principle

The hGH ELISA is based on the sandwich ELISA principle. Antibodies to hGH (anti-hGH) are prebound to the surface of the Microplate modules (Fig. 3).

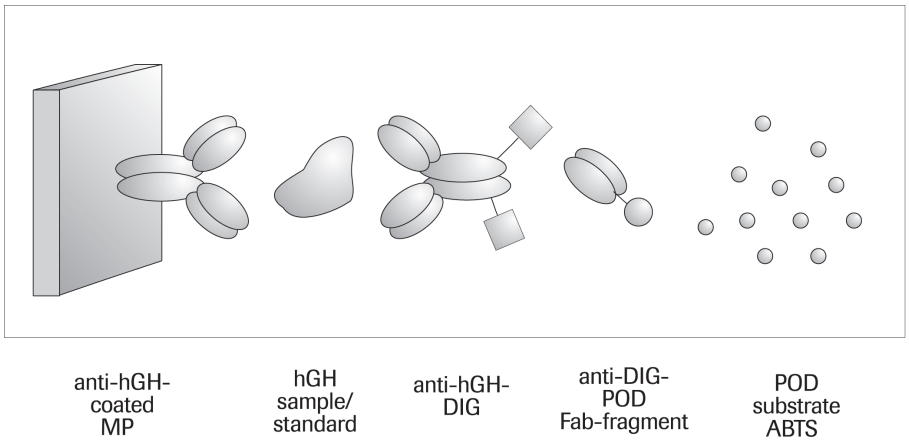

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Fig. 3: Test principle

① Following transfection of cells, the culture supernatant, which contains secreted hGH, is added to the wells of the Microplate modules. All hGH contained in the medium binds specifically to the anti-hGH antibodies bound to the microplate surface.

② Addition of digoxigenin-labeled antibody to hGH (anti-hGH-DIG) and binding to hGH.

③ Addition of an antibody to digoxigenin conjugated to peroxidase (Anti-DIG-POD) and binding to digoxigenin.

④ Addition of the ABTS peroxidase substrate.
- The peroxidase enzyme catalyzes the cleavage of the substrate yielding a colored reaction product.

⑤ The absorbance of the sample is determined using a microplate (ELISA) reader and is directly correlated to the level of hGH present in the medium supernatant.
- The sensitivity of the assay can be enhanced by using the ABTS peroxidase substrate with substrate enhancer.

Background Information

Human Growth Hormone (hGH; somatotropin) is a 22 kDa, 191 amino acid, polypeptide hormone synthesized and secreted by cells of the anterior pituitary. The hGH gene contains five exons and is the best characterized of five members of a gene family.

Gene expression in transfected mammalian cells is generally studied by linking a promoter sequence to mammalian or bacterial genes encoding an easily detectable reporter protein such as human growth hormone, chloramphenicol acetyltransferase (CAT), β-galactosidase (β-Gal), or luciferase. The hGH assay system differs from most of the other commonly used reporter proteins such as CAT, β-galactosidase, or luciferase in one important respect; hGH is a secreted protein and is measured using samples of culture medium supernatants, thus avoiding the necessity to lyse cells, allowing continuous monitoring of transient expression kinetics, and allowing the use of the cells for other purposes, such as RNA isolation. Additionally, hGH is a mammalian gene which may contribute to the apparent high stability of the hGH mRNA in most mammalian cells. The expression of reporter protein correlates directly to changes in the level of hGH mRNA.

Using the standard isotopic protocol, secretion of hGH protein into the culture medium supernatant can be monitored approximately 24 hours post transfection, depending on the cell type and the sensitivity of the assay used. Usually, hGH is quantified by an immunoradiometric assay (IRMA) resulting in a linear range of approximately 0.1 to 50 ng hGH/ml.

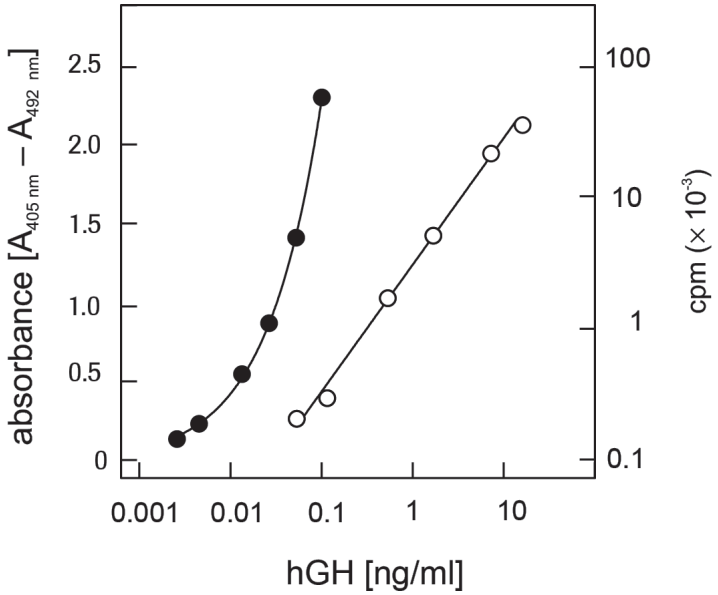

Fig. 4: Comparison of an immunoradiometric hGH assay (IRMA; O) versus the hGH ELISA (●). Various dilutions of hGH standard were assayed following a standard sandwich IRMA procedure using two monoclonal antibodies, each specific for a different and distinct epitope. One antibody is radiolabeled 125 while the other antibody is coupled to biotin. The incubation of the two antibodies and the sample containing hGH with an avidin-coated polystyrene bead allows the quantification of bound hGH. The non-isotopic hGH ELISA was performed as described. Note that in a non-logarithmic presentation, the hGH ELISA produces a linear calibration curve (Fig. 2).

Various vectors, such as pXGH5, pØGH containing polylinkers adjacent to the hGH sequence for insertion of promoters, are commonly used. In transiently transfected mouse L cells, the immunoradiometric hGH assay was described to be at least 10 times more sensitive than the CAT assay system. It should be noted that the hGH gene may not be entirely silent in terms of regulatory signals. In some cases, depending on the chosen cell line, the immunoradiometric hGH assay was reported to cross-react with rat growth hormone which is also secreted into the medium supernatant resulting in an elevated background signal.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DOTAP Liposomal Transfection Reagent	2 ml, 5 x 400 µl, 5 x 400 µg	11 202 375 001
CAT ELISA	1 kit, 192 tests	11 363 727 001

5.4. Trademarks

ABTS is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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