Product Code: 0805

# ESR1 Box 1.0

## Typing Kit

*In vitro* diagnostics disposal

## Instructions Manual

geneBOX

DESENVOLVIMENTO E PRODUÇÃO
DE TESTES DE DIAGNÓSTICO

Version 1.7; April 2010.



## Table of contents

## Presentation

This kit contains typing plates with dried primers mixes and PCR Master Mix for typing of polymorphisms 397 and 351 of Estrogen receptor gene (ESR1).

## Product Changes and Improvements

The ESR1 Box specificity and interpretation tables are constantly updated, to include new ESR1 mutations described. This product can also be improved in order to increase the yield of the specific PCR product.

The primers exchanged, added or modified, compared to the previous lot, are detailed in the table below.

| Tube | primers | Motivation |
|---|---|---|
| N/A | | |

## Quality Control

The specificity of each primer solution of the kit has been tested using DNA samples from Genebox. Positive and negative results were obtained for each mutation. The ESR1 Box 1.0 Typing kit has the Genebox quality warranty.



## ESR1 Box 1.0 Typing Kit Components

- **ESR1 typing plate**[+] (48 typings)

  2 plates (24 samples each) (Keep at -30/-15 ºC)

- **PCR Master Mix (With Taq DNA Polymerase)**

  2 X 340 µl (keep at -30 /-15ºC)

- **Plate sealers**

  2 PCR plate sealers

- **Instructions Manual**

  1 Instructions Manual

  [+] With dried specific primers pares.

**PCR Master Mix Components**

***Nucleotides***
Final concentration of each dNTP: 600 µM

***PCR Buffer***
Final concentration: 3,3x $NH_4$ Buffer; 2,0 mM $MgCl_2$ and 0,4 U/µl Taq DNA polymerase, pH 8.3.

***Glycerol***
Final concentration: 16,6%

***Cresol Red***
Final concentration: 300µg/ml

# PCR amplification protocol

## Reagents

- DNA Sample (100-200 ng/µl)
- PCR Master Mix
- $_{dd}H_2O$ (not supplied)

## DNA Extraction

For SSP typing highly pure DNA is needed. We recommend isolation of DNA using any extraction kit with CE marking, which guarantees an OD ratio 260/280 higher than 1.6 and a 100ng – 200 ng/µl DNA concentration.
Alternatively, the DNA can be extracted using trimethyammoium-bromide salts (DTAB/CTAB) or by salting out, dissolving it in TE Buffer. The same OD and concentration values should be assured.
DO NOT USE HEPARINISED BLOOD WITH THIS METHOD

## PCR Amplification

1. Spin briefly the DNA and Master Mix tubes.
2. Add:
   - **14 µl of PCR Master Mix,**
   - **30 µl of $ddH_2O$, and**
   - **4 µl of DNA sample (conc. 100-200 ng / µl)**

   to a 0,7 ml or 1,5 ml tube.
3. Vortex the tube vigorously for 15s.
4. Load **10 µl** of the mix into each tube of the plate (**4 primer pairs**).
5. Repeat the previous steeps for other DNA sample to complete the ESR1 typing plate.
6. Close the typing plate with a self-adhesive lid and put it in a 96 well thermo cycler.



## PCR Cycling Parameters

| Step | Temperature | time | Cycle |
|---|---|---|---|
| Denaturation | 96 ºC | 1 min | 1 |
| Denaturation<br>Annealing<br>Extension | 96 ºC<br>70 ºC<br>72 ºC | 25 sec<br>45 sec<br>30 sec | 5 |
| Denaturation<br>Annealing<br>Extension | 96 ºC<br>65 ºC<br>72 ºC | 25 sec<br>45 sec<br>30 sec | 21 |
| Denaturation<br>Annealing<br>Extension | 96 ºC<br>55 ºC<br>72 ºC | 25 sec<br>1 min<br>2 min | 4 |
| Extension | 72 ºC | 10 min | 1 |
| Keep<br>(optional) | 4 ºC | Infinite | 1 |

7. Keep the plate at 2-8 ºC after the PCR have finished.
8. Detect the PCR products with 2% agarose gel electrophoresis.

## Gel Electrophoresis protocol

### *PREPARING 2% AGAROSE GEL*

1. Dissolve **4 grams** of electrophoresis **grade agarose** powder in **200 ml** of **1X TAE buffer**.
2. Melt the agarose powder completely in a microwave oven.
3. Cool the heated agarose gel to **~ 50°C.**
4. Add at least 10 µl of **ethidium bromide**[++] (10 mg/ml) or **Sybr Safe**[TM] (100000 x concentrate) to the heated agarose. Stir until it is thoroughly incorporated.
5. On a balanced surface, set up a gel plate with **96 wells**.
6. Cast a **5mm** thick gel on the plate.
7. Allow the gel to settle.

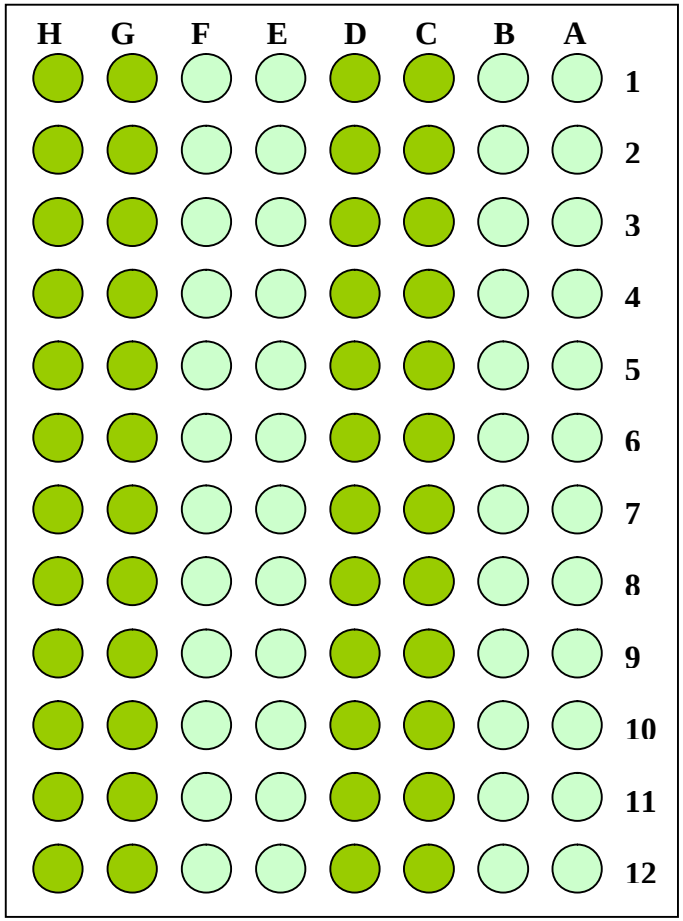
[++]**Caution**, this reagent is a strong mutagenic agent (read carefully its MSDS before using it).

### *GEL ELECTROPHORESIS*

1. Submerge the gel in 1X TAE buffer in a gel box.
2. Gently remove the caps to avoid splashing of PCR products.
3. Load 10 µl into each well on the gel.
4. Connect the electric leads and turn on the power supply (115V). Electrophoresis for ~ 20 minutes, or until 2/3 of the lane.
5. Transfer the gel onto a UV transilluminator, document the result by photography.
6. Use the ***result interpretation table (1 and 2)*** to interpret results.



## ESR1 Box 1.0 plate




## ESR1 Box 1.0 plate identification

| Position (Odd sample) | Position (Even sample) | Gene | Polymorphic site |
|---|---|---|---|
| A | E | ESR1 | 397 |
| B | F | ESR1 | 397 |
| C | G | ESR1 | 351 |
| D | H | ESR1 | 351 |

## Results Interpretation Table (1/2)

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 1 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 2 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 3 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 4 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 5 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 6 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

****Control primer pairs match with non-allelic sequences. The internal positive control primer pairs amplify segments of the gene PIC1, giving rise to 790 base pair fragments. In the presence of the specific band amplification the control band intensity often decreases.**



## Results Interpretation Table (2/2)

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 7 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 8 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 9 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 10 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 11 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

| Row | Well | | Gene | Polymorphism | Allele | Specific Band | Control band** |
|---|---|---|---|---|---|---|---|
| 12 | A | E | ESR1 | 397 | C | 203 | 790 |
| | B | F | ESR1 | 397 | T | 203 | 790 |
| | C | G | ESR1 | 351 | A | 157 | 790 |
| | D | H | ESR1 | 351 | G | 157 | 790 |
| | DNA 1 | DNA 2 | | | | | |
| | | | | | | | |

****The PCR reaction is only valid in the presence of control band or, in some cases, in the presence of the specific band. In the absence of the control band, please repeat the typing. If the PCR reaction results in fragments different from the specific or con troll band please do not consider, they are unspecific fragments.**

# Troubleshooting Guide

| PROBLEMS | POSSIBLE CAUSES | SUGGESTIONS |
|---|---|---|
| The control and specific bands are weak. | Concentration of DNA sample is too low. | Check DNA quality and concentration |
| | | Re-extract the DNA sample or try not add water into the PCR Mix |
| | | Repeat typing with a good quality DNA sample |
| | DNA polymerase inhibitors in the DNA sample | Re-purify the sample DNA |
| | | Repeat typing with a good quality DNA sample |
| Missing internal control bands in one or several lanes. | DNA polymerase inhibitors in the DNA sample. | Re-purify the sample DNA |
| | | Repeat typing with a good quality DNA sample |
| | Dried PCR amplification products | Check the plate sealing |
| | | Repeat the typing using a PCR MicroMat and/or overlay the PCR reaction mix with mineral oil |
| False negative of a specific band while the internal control appears normal | Degradation of DNA sample | Re-extract the DNA sample with fresh material |
| | | Repeat typing with a good quality DNA sample |
| More than two specific alleles are detected/ Ambiguous results | Excess of template DNA | Check DNA quality and concentration |
| | | Dissolve the DNA sample in $_{dd}$H2O in order to have the proper concentration |
| | | Repeat typing with a good quality DNA sample |
| | Contamination with previously amplified PCR products or with other DNA samples during the DNA extraction or PCR preparation steps | Clean the working area |
| | | Work in separated pre-PCR and post-PCR rooms |
| | | Keep different lab coats in pre-PCR and post-PCR rooms |
| | | Change protective gloves frequently |
| | | Repeat typing with a good quality DNA sample |
| Blurred bands | Degradation of DNA sample | Re-extract the DNA sample with fresh material |
| | | Repeat typing with a good quality DNA sample |
| | Excess of template DNA | Check DNA quality and concentration |
| | | Dissolve the DNA sample in $_{dd}$H2O in order to have the proper concentration |
| | | Repeat typing with a good quality DNA sample |
| | Electrophoresis Buffer Problems: wrong buffer or older buffer | Use a fresh recommended buffer |



# Precautions and Warnings

PCR amplification allows the amplification of small quantities of sample DNA in an exponential way. However, this is also true for foreign DNA, which can contaminate our PCR method. Consequently, special laboratory practices are necessary in order to avoid false positive amplifications. Bellow is listed Genebox recommendations to circumvent contaminations:

- Work in separated pre-PCR and post-PCR rooms.

- Laboratory workflow must be unidirectional, from pre-PCR to post-PCR area.

- Specific equipment for each working area must be used (sample preparation, amplification and preamplification).

- All equipment used in post-PCR should not leave this area.

- Use dedicated micropipettes, gloves and lab coats in each area.

- Use non talcum powder gloves (since talcum could inhibit the PCR reaction).

- Use filter tips in order to avoid cross contamination.

- Check regularly micropipettes, in order to ensure that they are accurate within 5 % of fixed volume.

- Use different micropipettes depending on the volume we wish to load.

- Check regularly thermocyclers, in order to ensure that they are accurate within 1% of fixed temperature.

- Open and close reagent vials carefully. After use, close vials and store at indicated temperatures.

- Do not use a kit after its expiration date.

- packaging material included within the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of the kit reagents.

- plastic material included within the kit is resistant under normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, the impossibility to use the kit.

- check suitability of DNA quantity and quality before use the kit.

General instructions for laboratory safety:

- do not eat, drink or smoke in laboratory work areas

- wear disposable gloves

- wear clean lab coats and eye protection

- wash hands thoroughly after handling specimens and test reagents

- clean the working area before and after kit handling.

- do not pipette by mouth.

# Technical Guide

## 1. DNA Quality and Concentration

For optimal results with the ESR1 Box 1.0 Typing Kit™ the quality of DNA is critical. Good quality DNA means an OD ratio 260/280 higher than 1.6 and the major portion of DNA should run higher than 9.4 kb on an agarose gel. Different quality and concentration values require DNA re-extraction.
The quantity of DNA should be 100ng – 200 ng/µl. Excess of DNA can cause unspecific amplification.
We recommend any DNA extraction kit which has CE marking, in order to obtain this highly DNA purity

## 2. Taq Polymerase

ESR1 Box 1.0 Typing Kit™ kits have been intensively tested with the Taq DNA Reagente 5 (Reagente 5, Lisboa, Portugal).

## 3. PCR Master Mix

For optimal results with the ESR1 Box 1.0 Typing Kit™ the use of master mix supplied is obligatory.

## 4. Amplification Procedure

At the end of PCR, examine the degree of evaporation and condensation of PCR reaction mixture. If there is more than 20% volume loss do not validate the results. In order to prevent this you should overlay the PCR reaction mixture with mineral oil or use a MicroMat. It is also a good practice to maintain QC records on the heating lid.
If the temperature of the heating lid is not high enough, it will cause condensation problems on the lid.

## 5. Thermal Cycler

We recommend the use of any thermocycler with the following characteristics:
- heating rate up to 2.5ºC/sec; cooling rate up to 1.5ºC/sec; temperature range 4-100ºC; temperature uniformity ±0.5ºC; heated lid up to 100ºC.

## 6. Validity

As specified in the package

**If your problems persist, do not hesitate to contact our technical support**

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# Guarantee

geneBOX - R&D Diagnostic Tests guarantees that the primers in ESR1 Box typing kit have the specificities given in the Results Interpretations Sheet/Tables of the product insert.

## 1. Typing plate

When stored at -20ºC, the dried primers are stable for 12 to 19 months from the date of manufacture (see lot validity in the package).
When stored at 4ºC, the dried primers are stable for 12 from the date of manufacture (see lot validity in the package).
At room temperature, the dried primers are stable for 3 to 4 weeks from the date of the reception.
When the sealer is removed the dried primers steal be stable for 2 days, maximum, in dried conditions.

## 2. PCR Master Mix

When stored at -20ºC, the PCR Master Mix is stable for 18 months from the date of manufacture (see lot validity in the package).
When stored at 4ºC, the Master mix is stable for 15 days from the date of the reception.
At room temperature, the master mix steal be stable for 3 days from the date of the reception.
The master mix should not be left or stored with the cap open.

## 3. DNA

Using extracted DNA from salting out or any kit procedure the samples should be stored at 4ºC or -20ºC. If you chose to freeze the samples you must avoid repeated cycles of heating/freezing, in order to preserve your sample stability.
The DNA samples stored in $dH_2O$ are stable for at least 2 to 4 weeks (at 4ºC) or 24 months (at -20ºC).
The DNA samples stored in buffer are stable for at least 12 months (at 4ºC) or 5 years (at -20ºC).

## Warranty

geneBOX - R&D Diagnostic Tests warrants its products to the client against defects in materials and contents under normal application. The company products under this warranty shall be replaced, at no charge, to the damaged client.

This warranty applies only to products that have been handled and stored in accordance with its recommendations/specifications.

The claims must be posted directly to geneBOX in writing and must be accompanied by a copy of the purchaser's invoice.

This product may not be reformulated, repacked or resold in any form without geneBOX - R&D Diagnostic Tests consent.



## Declaration of conformity

**Product Name:** ESR1 Box

**Product Number:** GB.08.05

**Intended use:** Genotyping of Estrogen receptor gene.

**Manufacturer:**

geneBOX - R&D Diagnostic Tests,
Biocant – centro de inovação em biotecnologia
núcleo 4, lote 3
3060-197 Cantanhede,
Portugal

We, geneBOX - R&D Diagnostic Tests, hereby declare that this product, to which this declaration of conformity relates, is in conformity with the following standards and other normative documents ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices as transposed into the national laws of the Member States of the European Union.

The technical file of the product is maintained at geneBOX - R&D Diagnostic Tests, Biocant Park, Parque tecnológico de Cantanhede, 3060-197 Cantanhede, Portugal.

Sandra Balseiro
Technical Director

# Material Safety Data Sheet (MSDS) (1/3)

## geneBOX - R&D Diagnostic Tests™ PCR-SSP Kits

### geneBOX ™ PCR-SSP typing products

This Material Safety Data Sheet (MSDS) applies to all geneBOX - R&D Diagnostic Tests SSP™ typing kits

### 1. Chemical products and company identification

| | |
|---|---|
| Date of Issue: | May 2010 |
| Product group: | geneBOX™ PCR-SSP Typing Products |
| Manufacturer: | geneBOX - R&D Diagnostic Tests,<br>biocant – centro de inovação em biotecnologia<br>núcleo 4, lote 3<br>3060-197 cantanhede, portugal |
| tel/fax: | +351 231 410 946/ +351 231 410 947 |
| e-mail: | info@genebox.com |

### 2. Composition and reagents information

| Component | Chemical | Common Name |
|---|---|---|
| Plate | Deoxyribonucleic acid | Oligonucleotide |
| | Cresol Red | |
| PCR Master Mix | Deoxyribonucleotides | Nucleotides |
| | $NH_4$ Buffer | |
| | Magnesium chloride | MgCl2 |
| | Cresol Red | |
| | Glycerol | Glycerine |

### 3. Physic-chemical properties:

| Components | Appearance | Colour | Odour |
|---|---|---|---|
| Plate | dried, in plate wells | Red | none |
| Master Mix | liquid | Pink/red | none |

### 4. Toxicological information

| Chemical | Toxicities |
|---|---|
| Glycerol | LD50= oral 4090 mg/kg (mouse) |
| | LD50= oral 12600 mg/kg (rat) |
| | LD50= oral 1480 mg/kg (human) |

### 5. Stability and reactivity

**Conditions to avoid:** Heat and moisture.
**Incompatibilities:** Strong oxidizing agents, strong bases.



# Material Safety Data Sheet (MSDS) (2/3)

### 6. Personal protection.

**Hand protection:** Wear appropriate chemically resistant gloves.
**Eye protection:** Chemical safety goggles are recommended.
**Skin protection:** Wear laboratory coat.

### 7. Handling and storage

**Handling:** Avoid substance contact.
**Storage:** Protect from light. Store at temperature indicated on package.
**Package Damage:** reject damaged components.

### 8. Hazards

**Master Mix Components:** may be harmful by **inhalation, ingestion or skin absorption**. May cause eye and skin irritation. Material is irritating to mucous membranes and upper respiratory tract. **Ingestion** of large amounts can cause stomach pains, vomiting or diarrhoea.

### 9. First aid measures

**In case of eye contact:** Immediately flush eyes with large amounts of water for at least 15 minutes. Call a physician.
**In case of skin contact:** Immediately wash skin with soap and large amounts of water. Wash contaminated clothing before re-use.
**In case of ingestion:** Wash out mouth with water provided person is conscious. Call a physician if needed.
**In case of inhalation:** remove to fresh air, if not breathing give artificial respiration. If breathing difficult, give oxygen. Call a physician.

### 10. Fire fighting measures

**Extinguishing media:** Water, carbon dioxide, dry chemical powder or appropriate foam.
**Extinguishing media NOT to use:** None are known.
**Special exposure hazards:** May emit toxic fumes of carbon dioxide, carbon monoxide, nitrogen, phosphorus, hydrogen chloride, and hydrogen gas under fire conditions.
**Special fire-fighting equipment:** When large amounts of substances are released work only with self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

### 11. Accidental release measures

**Personal Precautions:** Avoid substance contact. No further requirements.
**Cleaning Method:** Clean up affected area. No further requirements.

## Material Safety Data Sheet (MSDS) (2/3)

### 12. Ecological information
No data available.

### 13. Waste disposal information
Waste disposes in accordance with all applicable regulations (the disposals should be incinerated).

### 14. Transport information
During transportation the temperature could not exceed 25ºC.
Transportation should not exceed 3 days.

### 15. Other information
The above information is based on our current level of knowledge, but does not purport to be all-inclusive and shall be used only as a guide. *geneBOX - R&D Diagnostic Tests* shall not be held liable for any damage resulting from handling or from contact with the above products.

**If your problems persist, do not hesitate to contact our technical support**

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## References

1. Wedren S, Lovmar L, Humphreys K, Magnusson C, Melhus H, Syvanen AC, Kindmark A, Landegren U, Fermer ML, Stiger F, Persson I, Baron J, Weiderpass E. Oestrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: a case control study. *Breast Cancer Res.* 2004; 6: 437-449.

2. Shearman AM, Karasik D, Gruenthal KM, Demissie S, Cupples LA, Housman DE, Kiel DP. Estrogen receptor beta polymorphisms are associated with bone mass in women and men: the Framingham Study. *J Bone Miner Res.* 2004; 19: 773-781.

3. Schuit SC, van Meurs JB, Bergink AP, van der Klift M, Fang Y, Leusink G, Hofman A, van Leeuwen JP, Uitterlinden AG, Pols HA. Height in pre- and postmenopausal women is influenced by estrogen receptor alpha gene polymorphisms. *J Clin Endocrinol Metab.* 2004; 89: 303-309.

4. van Meurs JB, Schuit SC, Weel AE, van der Klift M, Bergink AP, Arp PP, Colin EM, Fang Y, Hofman A, van Duijn CM, van Leeuwen JP, Pols HA, Uitterlinden AG. Association of 5' estrogen receptor alpha gene polymorphisms with bone mineral density, vertebral bone area and fracture risk. *Hum Mol Genet.* 2003; 12: 1745-1754.

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