# PRONTO® GAUCHER

## 370Rec Allele Identifier

**Instructions for Use**

REF **9915**

4 Tests

PRONTO DIAGNOSTICS

+2°C +8°C

IVD CE

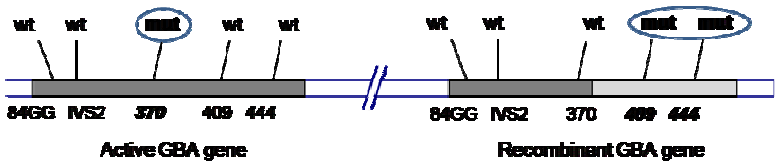
## BACKGROUND

The human glucocerebrosidase gene (GBA) resides on chromosome 1q21. A pseudogene of GBA, located 16 Kb downstream to the gene, shares 96% DNA sequence homology with the active GBA gene.

A few recombinant alleles, which possibly arose as a result of a chromosomal rearrangement involving the functional gene and the pseudogene, such as RecTL and RecNci, were previously described.

A few point mutations that sometimes appear in the active gene, i.e., D409H, L444P and others, are part of the normal sequence of the pseudogene and consequently when both mutations (D409H and L444P) are detected with the Pronto® Gaucher kit (cat. number 9900) the presence of a recombinant allele must be suspected. If, upon using the Pronto® Gaucher kit, one simultaneously detects **N370S, D409H** and **L444P** in the same sample, the presence of the **370Rec** recombinant allele must be assumed. Since the N370S point mutation is absent from the GBA Pseudogene the 370Rec allele is probably an outcome of recombination event which involved an active GBA gene that harbored the N370S mutation with one of the other Rec alleles.

**Structural model for the 370Rec allele**




In the presence of 370Rec allele, a person who is homozygous for N370S will be typed by the Pronto Gaucher kit as a heterozygote for this mutation. This 370Rec Allele Identifier package contains a specific amplification mix, which amplifies the N370S genomic DNA region only on the active gene and not on the GBA pseudogene. Only by using this specific amplification mix, is the correct N370S genotype identification in 370Rec carriers possible.

Please Note: When using the Pronto® Gaucher Screen kit (cat. number 9900-1), which does not detect the D409H mutation, the presence of the 370Rec allele should be suspected in cases where both N370S and L444P are detected. If an N370S/N result was obtained using this N370S mix, an additional test for the D409H mutation should be considered in order to distinguish between the two following genotypes: a compound heterozygote 370/444 and 370Rec/N.



## CONTENTS OF THE PACKAGE

Gaucher N370S Amplification mix……1 x vial (0.2 mL)
PRONTO® Gaucher N370S strip……1 x pouched strip
Detection strip ……1 x Streptavidin-coated strip

*Other required components – Use from Pronto® Gaucher kit

**Please refer to Pronto® Gaucher kit manual for storage and stability, additional materials required and disclaimer.**

## ASSAY PROCEDURE

### 1 DNA AMPLIFICATION

1. **Dispense** 2 µL template DNA (from an initial concentration of about 150 ng/µL) to a thermoplate well or tube.
2. **Prepare** a Master Mix in a sterile vial, according to the volumes indicated in the table below, plus one spare reaction volume. Add the Taq DNA polymerase to the amplification mix shortly before dispensing the mix. Gently mix by pipetting in and out several times.

**Master mix**

| Solution | Volume for one sample |
|---|---|
| Gaucher N370S Amplification mix | 22.5 µL |
| Taq DNA polymerase (5 u/µL) | 0.5 µL |

The following Taq DNA polymerases were validated for use with this procedure (lacking 3' → 5' exonuclease activity):

- PHARMACIA Cat. # 27-0799
- SIGMA Cat. # D-1806
- ROCHE Cat. # 1-146-165
- PROMEGA Cat. # M-1661
- BIOLINE Cat. # M95801B
- PERKIN ELMER Cat. # M801-0060
- BIOLABS Cat. # M0267S
- ProntoTaq Cat. # 9101

3. **Dispense** 23 µL master mix to each well or tube.
4. **Add** one drop of ColoRed™ oil to each well. Do not touch the wells with the tip of the oil bottle. Even when using a thermocycler with a hot lid, it is essential to use oil.
5. **Place** the thermoplate well or tube in a thermocycler previously programmed with the following protocol:

**Cycling protocol**

| | | | |
|---|---|---|---|
| 1. | 94ºC | 2 minutes | |
| 2. | 94ºC | 30 seconds | 35 cycles |
| 3. | 60ºC | 30 seconds | |
| 4. | 72ºC | 45 seconds | |
| 5. | 72ºC | 5 minutes | |

6. To verify amplification, subject 5 µL of the amplified product to electrophoresis in a 2% agarose gel.

   One **380bp** amplified fragment encompassing the N370S point mutation should be visible.



**Limitation of the test:**

Different Taq DNA polymerases and thermocyclers may influence the amplification yield dramatically. It is recommended to use a validated Taq DNA polymerase and a calibrated thermocycler.

## 2 POST-AMPLIFICATION TREATMENT

**Perform** the post-amplification treatment according to the instructions of the Pronto® Gaucher kit manual (page 7).

## 3 PRIMER EXTENSION REACTION

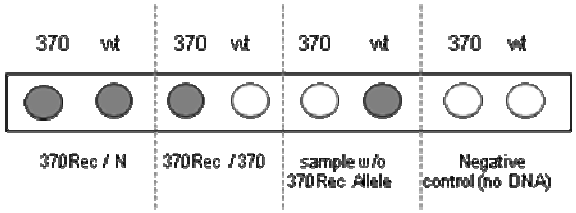
1. **Program** the thermocycler according to the protocol in the Pronto® Gaucher kit manual (page 8).
2. **Take** a PRONTO® Gaucher N370S Strip out of its pouch. Notice the color at the bottom of the wells. For each sample tested, use one pink well (mut) and one blue well (wt). Mark the strip with the ID numbers of your test.
3. Each strip is sufficient for testing four samples. **Cut** the appropriate number of wells and return the unused portion to the pouch. Seal the pouch immediately with its desiccant inside.
4. **Dispense** 8 µL of each post-amplification treated DNA into two wells.
5. **Add** one drop of ColoRed™ oil to each well.
6. **Turn on** the thermocycler and start the cycling protocol (page 8).
7. When the thermal cycling is complete, you can proceed to the ELISA assay, or store the reaction products in the refrigerator and carry out the ELISA steps within 24 hours.

# 4 TRANSFER TO THE ELISA STRIP

1 Take the ELISA detection strip out of its pouch.
2 Cut the appropriate number of wells and return the unused portion to the pouch. Seal the pouch immediately with its desiccant inside.
3 Place the wells in an empty ELISA strip on a detection plate frame and follow the instructions in Pronto® Gaucher kit manual on pages 10-12.

**For validation and interpretation of the results please refer to Pronto® Gaucher kit manual and see Fig. 1.**

**Fig. 1**: Genotype assignment according to visual inspection of test results




Note: In case of an heterozygote result, the compound genotype of recTL (or 409/444) and N370S cannot be excluded. The patient's clinical picture and further tests are required to conclude the diagnostic process.



For troubleshooting guide, please refer to our website:
www.prontodiagnostics.com/ts



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