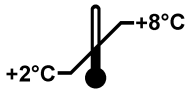
PRONTO® Tay-Sachs Screen kit

For the detection of the following mutations in Ashkenazi and North African Jews in the HEXA gene:

1278insTATC
G269S (805G>A)
IVS12+1G>C
R170Q
IVS5 -2A>G
F304del

Instructions for Use

REF 9981



Tests

PRONTO DIAGNOSTICS

CE IVD

INTENDED USE

The Pronto® Tay-Sachs Screen kit is a single nucleotide primer extension ELISA procedure, intended for the qualitative *in vitro* detection of the following six mutations in the HEXA gene: c.1278insTATC, c.IVS12+1G>C, p.G269S, p.R170Q, c.IVS5 -2A>G and p.F304del in amplified human DNA.
For *in vitro* diagnostic use.

BACKGROUND

Tay-Sachs disease is a fatal, autosomal recessive, neurodegenerative disorder caused by mutations in the HEXA gene, located on the human 15q23-q24 chromosome. It is characterized by normal motor development in the first few months of life followed by progressive weakness and loss of motor function, starting at 2-6 months of age, decreased social interaction, seizures and blindness, leading to total debilitation. Death typically occurs between 2 and 5 years of age. Tay-Sachs disease is caused by excess storage of GM2 ganglioside in lysosomes due to a deficiency of the isoenzyme hexosaminidase (hex A).
The carrier frequency of mutations that cause Tay-Sachs varies with ethnicity and is significantly elevated in both the Ashkenazi Jewish and Moroccan Jewish populations. The carrier frequency in Ashkenazi Jews is approximately 1 in 30 and about 1 in 110 in Jews of Moroccan extraction. A frequency of approximately 1 in 280 has been observed among other Israeli Jewish populations. The overall carrier frequency for Tay-Sachs disease in non-Jewish individuals is 1 in 300, with a higher carrier rate in French-Canadian or Cajun descent. Three mutations in the HexA gene account for approximately 98% of all Tay-Sachs mutations found in the Ashkenazi Jewish population: c.1278insTATC 81%, c.IVS12+1G>C 15% and p.G269S 2%. Among Moroccan Jews seven mutations in the HEXA gene were identified, three of which were most frequent: p.F304del - an in-frame deletion of one of two adjacent phenylalanine codons 304 and 305 with an allele frequency of 40%, p.R170Q 35% and c.IVS5 -2 A>G 10%.

In addition to general population screening, Tay-Sachs DNA testing is strongly recommended for individuals with ambiguous or positive HexA enzyme test results.

REFERENCES

1. Akerman BR, Natowicz MR, Kaback MM, Loyer M, Campeau E, Gravel RA. Novel mutations and DNA-based screening in non-Jewish carriers of Tay-Sachs disease. *Am. J. Hum. Genet*., 60:1099-106 (1997)
2. Kaback M, Lim-Steele J, Dabholkar D, Brown D, Levy N, Zeiger K. Tay-Sachs disease-carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. *J.A.M.A.,* 270:2307-15 (1993)
3. Kaback MM. Population-based genetics screening for reproductive counseling: the Tay-Sachs experience. *Eur. J. Peds.* 159:S192-5 (2000).
4. Navon R, Argov Z, Frisch A. Hexosaminidase A deficiency in adults. *Am. J. Med. Genet.*, 24:179-96 (1986)
5. American College of Obstetricians and Gynecologists (ACOG) Committee on Genetics. Pub. no. 162 (1995)
6. Okada S and O'Brien JS. Tay-Sachs disease: generalized absence of a beta-D-N- acetylhexosaminidase component. *Science* ,165:698-700(1969)
7. Karpati M, Peleg L, Gazit E, Akstein E, Goldman B: A novel mutation in the HEXA gene specific to Tay-Sachs disease carriers of Jewish Iraqi origin. *Clin. Genet.* 57:398-400 (2000).
8. Kaufman M, Grinshpun-Cohen J, Karpati M, Peleg L, Goldman B, Akstein E, Adam A, Navon R. Tay-Sachs disease and HEXA mutations among Moroccan Jews. *Hum. Mut.* 10:295-300 (1997).
9. Drucker L, Proia R, Navon R. Identification and rapid detection of three Tay-Sachs mutations in the Moroccan Jewish population. *Am. J. Hum. Genet*.; 51(2):371-7 (1992 Aug).

WARNINGS & PRECAUTIONS

- Reagents supplied in this kit may contain up to 0.1% sodium azide that is toxic if swallowed. Sodium azide has been reported to form explosive lead or copper azides in laboratory plumbing. To prevent the accumulation of these compounds, flush the sink and plumbing with large quantities of water.

- TMB Substrate solution is an irritant of the skin and mucous membranes. Avoid direct contact.
- In addition to reagents in this kit, the user may come in contact with other harmful chemicals that are not provided, such as ethidium bromide and EDTA. The appropriate manufacturers' Material Safety Data Sheets (MSDS) should be consulted prior to the use of these compounds.

ASSAY OVERVIEW

The PRONTO® procedure detects predefined polymorphisms in DNA sequences, using a single nucleotide primer extension ELISA procedure.

1 **TARGET DNA AMPLIFICATION:** The DNA fragments that encompass the tested mutations are amplified. This amplified DNA is the substrate for the primer extension reaction.

2 **POST-AMPLIFICATION TREATMENT:** The amplified DNA is treated to inactivate free unincorporated nucleotides, so that they will not interfere with the primer extension reaction.

3 **PRIMER EXTENSION REACTION:** A single-nucleotide primer extension reaction is carried out in a 96-well thermoplate. Each well contains a 5'-labeled primer that hybridizes to the tested DNA next to the suspected mutation site, and a single biotinylated nucleotide species, which complements the nucleotide base at the tested site. Each post-amplification treated sample is tested in two wells per mutation: the first well of each pair tests for the presence of the mutant allele (*mut*), while the second well tests for the presence of the normal allele (*wt*). The biotinylated nucleotide will be incorporated to the primer in the course of the reaction or not added, depending on the tested individual's genotype.

4 **DETECTION BY ELISA:** The detection of the biotinylated primers is carried out by an ELISA procedure: The biotin-labeled primers bind to a streptavidin-coated ELISA plate and are detected by a peroxidase-labeled antibody (HRP) directed to the 5' antigenic moiety of the primer.
A peroxidase reaction occurs in the presence of TMB-Substrate.

5 **INTERPRETATION OF THE RESULTS:** The results are determined either visually (substrate remains clear or turns blue) or colorimetrically.

DISCLAIMER

- Results obtained using this kit should be confirmed by an alternative method.
- Confirmed results should be used and interpreted only in the context of the overall clinical picture. The manufacturer is not responsible for any clinical decisions that are taken.

The user of this kit should emphasize these points when reporting results to the diagnosing clinician or the genetic counselor.

CONTENTS OF THE KIT

Tay-Sachs Screen Amplification Mix	1 vial	(0.5 mL)
ProntoTaq™	1 vial	(20 µL)
PRONTO® Buffer 2	1 bottle	(3 mL)
Solution C	1 vial (yellow cap)	(130 µL)
Solution D	1 vial (red cap)	(100 µL)
ColoRed™ Oil	1 dropper bottle	(13 mL)
Assay Solution	1 bottle (green solution)	(100 mL)
Wash Solution (conc. 20x)	1 bottle	(100 mL)
HRP Conjugate	1 vial	(450 µL)
TMB Substrate	1 bottle	(40 mL)
PRONTO® Tay-Sachs Screen Plates	3 individually pouched plates	
Detection Plates	3 Streptavidin-coated ELISA plates	

STORAGE AND STABILITY

- Store the ProntoTaq™ at -20°C.
- Keep the kit at 2-8ºC; Do not freeze.
- Do not use the kit beyond its expiration date (marked on box label). Stability is maintained even when components are re-opened several times.
- Minimize the time reagents spend at room temperature.
- This kit has been calibrated and tested as a unit; **do not mix reagents from kits with different lot numbers.**

ADDITIONAL MATERIALS REQUIRED

- Deionized water (about two liters per kit)
- Thermowell plate or tubes (thin wall) for the post-amplification treatment
- Sterile pipette tips
- Troughs/reagent reservoirs - for use with the detection reagents
- Thermocycler for a 96-well microplate
- Multichannel pipettes (5-50 µL and 50-200 µL)
- Positive displacement pipettes (1-5 µL, 5-50 µL, 50-200 µL & 200-1,000 µL)
- Filtered tips
- ELISA reader with 620 nm filter
- Polaroid camera and color film to record results (optional)
- Automated microtiter plate washer or squirt bottle
- Vortex mixer
- Timer

ASSAY PROCEDURE

1- DNA AMPLIFICATION

1. **Dispense** 2 µL template DNA (from an initial concentration of about 150 ng/µL) to a Thermowell plate 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 ProntoTaq™ to the Master Mix shortly before dispensing the mix. Gently mix by pipetting in and out several times.

PCR Master mix

Solution	Volume for one sample
Amplification Mix Tay-Sachs Screen	13.0 µL
ProntoTaq™	0.3 µL

3. **Dispense** 13 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 recommended to use oil.

5. **Place** the Thermowell plate or tube in a thermocycler previously programmed with the following protocol:

Cycling protocol

1.	95ºC	2 minutes	
2.	95ºC	15 seconds	35 cycles
3.	62ºC	30 seconds	
4.	72ºC	10 seconds	
5.	72ºC	2 minutes	

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

Sizes of amplified fragments:

Gene	Mutation	Fragment size
HEXA	c.1278insTATC c.IVS12+1G>C	600 bp
	p.G269S	293 bp
	p.R170Q	549 bp
	c.IVS5 -2A>G	357 bp
	p.F304del	410 bp

Limitation of the test:

Different thermocyclers may influence the amplification yield dramatically. It is recommended to use a calibrated thermocycler.

2 POST- AMPLIFICATION TREATMENT

1 **Prepare** a post-amplification treatment mix shortly before use. Combine in a single test tube the volumes appearing in the following table, multiplied by the number of tested samples, plus one spare volume.

Post-Amplification Mix

Solution	Volume for one sample
PRONTO® Buffer 2	93.0 µL
Solution C	4.0 µL
Solution D	3.0 µL

2 **Mix** gently by pipetting this solution in and out five times. Do not vortex.

3 **Add** 100 µL of the post-amplification mix into each Thermowell or tube containing 10 µL of each amplified DNA sample.
Ensure that the solution you add becomes well mixed with the DNA sample by pipetting.

4 **Add** one drop of ColoRed™ oil to each tube. Do not touch the tube with the tip of the oil bottle. Even when using a thermocycler with a hot lid, it is essential to use oil.

5 **Incubate** for 30 minutes at 37ºC, then for 10 minutes at 95ºC in a thermocycler.

If not used immediately, the treated sample can be kept at 2-8ºC for a maximum of four hours.

3 PRIMER EXTENSION REACTION

1 **Program** the thermocycler as follows:

Cycle	Temperature	Time
20 cycles:	94ºC	30 sec.
	62ºC	20 sec.
End:	18-25ºC - Cool down to room temperature	

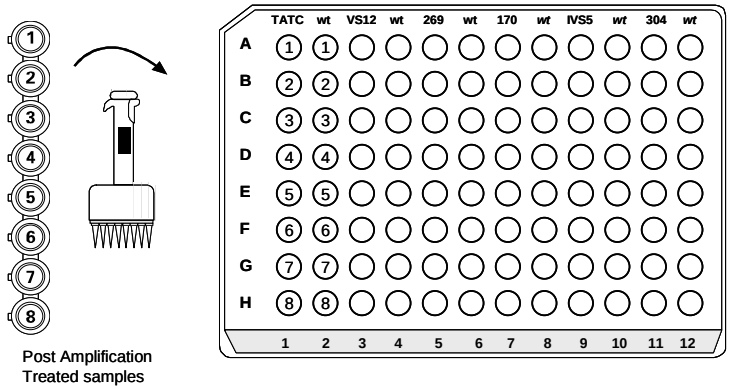
2 **Take** a PRONTO® Plate out of its pouch. Notice the color at the bottom of the wells. For each mutation tested, use a pink well (*mut*) and a blue well (*wt*). Mark the plate with the ID numbers of your test.
If you intend to use less than a full plate, you can cut the plate and return the unused portion to the pouch. If you do this, seal the pouch immediately with its desiccant card inside.

3 **Dispense** 8 µL of post-amplification treated DNA into the first twelve wells in row A (see Fig. 1). Continue with the remaining samples. It is possible to transfer up to eight samples simultaneously using a multichannel pipette.

Ensure that the solution is at the bottom of each well by inspecting the plate from below. Be sure that the well does not contain air bubbles.

Note – If you wish to test only the three North African mutations (170, IVS5 and F304) cut as many of the relevant rows as needed from the PRONTO® plate and store the remainder of the plate in the aluminum pouch. Dispense 8 µL of post-amplification treated DNA into the six wells and continue as instructed.

Figure 1: Scheme for dispensing Post-Amplification Treated DNA samples into the PRONTO® Tay-Sachs Screen Plate



Recommendation:
Use a new set of tips for each column. Alternatively use the same set of tips, but do not touch the bottom of the wells.

4 **Tilt** the plate and add one drop of ColoRed™ Oil to each well. Do not touch the well with the tip of the oil bottle. Even when using a thermocycler with a hot lid, it is not essential to use oil.
5 **Turn on** the thermocycler and start the cycling protocol.
6 When the thermal cycling is complete, you can proceed to the ELISA, or store the reaction products in the refrigerator and carry out the visualization steps within 24 hours.

4 ELISA ASSAY - COLOR DEVELOPMENT

The ELISA consists of the following steps:

- **Binding** the biotinylated primer to the Streptavidin-coated plate.
- **Washing** away the unbound primer.
- **Incubating** with the HRP conjugate.
- **Washing** away the unbound conjugate.
- **Incubating** with the TMB Substrate (color development).

The results of this assay can be determined in one of two ways:

a **Visually**: by monitoring the development of the blue color.

or

b **Colorimetrically:** by measuring the absorbance using an ELISA reader at a wavelength of 620 nm.

PREPARATION

- All components used in the detection step should reach room temperature before starting the assay.
- **Dilute** the 20x Wash Solution to 1x with deionized water.
  Dilute solution may be kept at 18-25 ºC for up to one month.
- **Peel** off the plastic cover of the Detection Plate. Mark the side of the plate with the kit name and test number.
- **Place** the PRONTO® Plate and the Detection Plate side by side, oriented in the same direction (see Fig. 2).

DETECTION BY ELISA

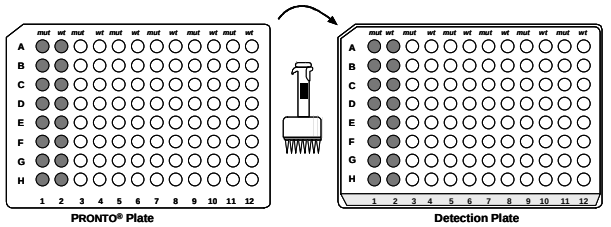
1 **Fill** a reagent reservoir /trough with the green colored Assay Solution. About 11 mL will be required for a 96-well plate.

2 Using a multichannel pipette, **add** 100 µL of Assay Solution to the bottom of each well in column 1 of the PRONTO® Plate. Gently mix by pipetting in and out 3-4 times.

3 **Without changing tips, transfer** 100 µL from each well in this column to the first column in the Detection Plate (see Fig. 2).

Ensure that the solution at the bottom of all wells of the PRONTO® *plate has turned green by inspecting them from below*.

Figure 2: Transferring the primer extension products from the PRONTO® Plate to Detection Plate.



4 **Repeat** this procedure, using a new set of tips for each column. It is essential to maintain the order of the samples.
10 µL of oil carried over or 10 µL of the sample left behind will not significantly affect the detection process.

5 **Incubate** for 10 minutes at room temperature (18-25ºC).

6 While the incubation takes place, dilute the Conjugated HRP 1:100 in assay solution (green solution). About 11 mL are needed for a 96-well plate. This solution must be freshly prepared each time the test is run.

7 **Empty** the plates, wash four times with 350 µL 1x Wash Solution. Ensure that the plates are dry after the last wash step.

8 With a multichannel pipette add 100 µL freshly-diluted Conjugated HRP to all the wells.

9 **Incubate** for 10 minutes at RT.

10 **Wash** as in step 7.

11 Add 100 µL TMB substrate to each well with a multichannel pipette and incubate for 15 minutes at RT (18-25°C) until the blue color appears sufficiently strong.

12 **For Visual Detection:** Results may be documented by a standard Polaroid camera with color film (for example, Fuji FP-100C).

13 **For Colorimetric Detection**: Agitate the plate gently to homogenize the color in the wells. Read the results in an ELISA reader using a 620 nm filter (singe wavelength setting).

VALIDATION OF THE RESULTS

For Visual Detection:

For every mutation site tested, at least one of the wells should develop a deep blue color. Otherwise, the results are invalid for the relevant mutation (see Fig. 3).

For Colorimetric Detection:

For every mutation site tested, at least one of the two wells should yield an O.D. ≥ 0.50 reading.

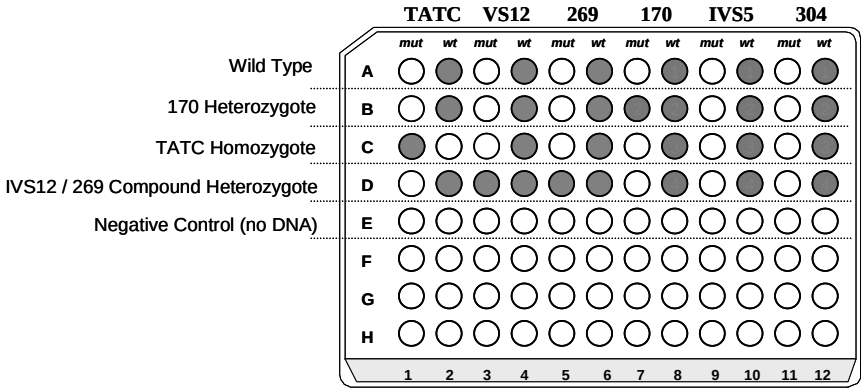
INTERPRETATION OF RESULTS

Important: Heterozygous or homozygous mutant results should be confirmed by retesting. It is recommended to repeat the test with newly extracted DNA.

Criteria for Visual Interpretation

A deep blue color indicates a positive signal, while negative signals appear as a clear or pale blue-colored well (see Fig. 3).

Figure 3: Examples of genotype assignment according to visual inspection of test results



Criteria for Colorimetric Interpretation (O.D. 620)

The genotype of each sample is determined according to two criteria:

1. The O.D. values of the *mut* and *wt* wells.
2. The ratio of *mut / wt* O.D. values.

Calculate the *mut / wt* ratios by dividing the signal of the *mut* well by the signal of the *wt* well.

Identify the correct genotype using the table below:

Genotype	*mut* well	*wt* well	mut/wt ratio
Normal	O.D. ≤ 0.35	O.D. ≥ 0.5	ratio ≤0.5
Heterozygote	O.D. ≥ 0.5	O.D. ≥ 0.5	0.5 < ratio < 2.0
Homozygote	O.D. ≥ 0.5	O.D. ≤ 0.35	ratio ≥ 2.0

! Samples with values not included in the above table are considered indeterminate and should be retested.

PRONTO® Tay-Sachs Screen PROCEDURE SUMMARY

DNA EXTRACTION: from human whole blood, using a validated method.

DNA AMPLIFICATION:
Volumes per reaction: 2 µL template DNA **+** 13 µL Amplification Mix + 0.3 µL ProntoTaq™
Cycling protocol:
95°C 2 min→35 cycles of {95 °C 15 sec. / 62 °C 30 sec. / 72 °C 10 sec.} →72 °C 2 min.

POST-AMPLIFICATION PROCEDURE:

▪ Volumes per reaction:	PRONTO® Buffer 2	93.0 µL
	Solution C	4.0 µL
	Solution D	3.0 µL

- **Pipette** in and out to mix.
- **Add** 100 µL into each well containing 10 µL amplified product, mix well.
- **Add** one drop of ColoRed™ oil.
- **Incubate** 30 minutes at 37 °C, then 10 minutes at 95 °C.

PRIMER EXTENSION REACTION:
- **Dispense** 8 µL of each Post-Amplification treated DNA into twelve wells of the PRONTO® Plate.
- **Add** one drop of ColoRed™ oil.
- **Start** the cycling protocol:
- 20 cycles of {94 °C 30 sec. / 62 °C 20 sec.} →Cool.

Insert the PRONTO® Plate in the thermocycler when the temperature is 90 °C.

DETECTION:
- Add 100 µL Assay Solution to each well in the PRONTO® plate and mix.
- Transfer 100 µL from each well of the PRONTO® Plate to the respective position in the Detection Plate. Incubate 10 minutes at RT.
- Empty the wells and wash four times with 350 µL of 1x Wash Solution.
- Add 100 µL 1:100 Conjugate HRP to every well; incubate for 10 minutes at RT.
- Wash the wells again.
- Add 100 µL Substrate to each well; incubate at RT for 15 minutes.

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

Produced by Pronto Diagnostics Ltd.
Kiryat Atidim
Building 3, 3rd floor
POB 58129
Tel Aviv 6158002
Israel
Tel: +972.73.2126155
Fax: +972.73.2126144

Customer Service: E-Mail: info@prontodiagnostics.com

Authorized EU Representative:
MedNet GmbH,
Borkstrasse 10, 48163 Münster,
Germany
Tel: +49.251.32266-0
Fax: +49.251.32266-22

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