PGX-HIV StripAssay ™

REF 4-710 / (incl. 4-710-AM)

Σ 20 Tests

2-8°C

IVD

CE

1.	**Lysis Solution**	50 ml	
2.	**GEN^{X}TRACT Resin**	5 ml	
	Resuspend each time immediately before removing an aliquot.		⚠
3.	**Amplification Mix** *(yellow cap)*	500 µl	
4.	**Taq Dilution Buffer** *(transparent cap)*	500 µl	
5.	**DNAT** *(blue cap)*	1.5 ml	✖ R 36/38
6.	**Typing Trays**	3	
7.	**Teststrips**	20	
8.	**Hybridization Buffer**	25 ml	
9.	**Wash Solution A** *(white cap)*	80 ml	
10.	**Conjugate Solution**	25 ml	
11.	**Wash Solution B**	80 ml	
12.	**Color Developer**	25 ml	

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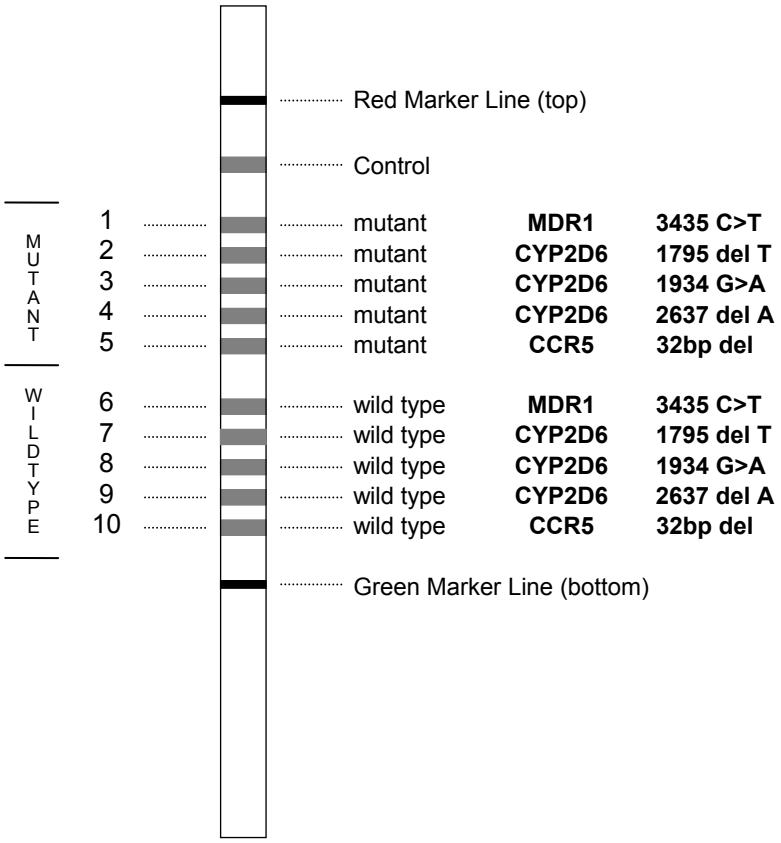


Fig. 1: Teststrip Design

Note: Teststrip is not drawn in real size and must not be used for interpretation of results!

Instructions for use

I. INTENDED USE

Assay for the identification of genotypes associated with response to HIV highly active anti-retroviral therapy (HAART) based on polymerase chain reaction (PCR) and reverse-hybridization.

II. METHODOLOGY

The procedure includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines (Fig. 1). Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

The assay covers 5 polymorphic loci: MDR1 3435 C>T, CYP2D6 1795delT (2D6*6), CYP2D6 1934 G>A (2D6*4), CYP2D6 2637delA (2D6*3), CCR5 32bp deletion.
Further genetic information is available at OMIM Online Mendelian Inheritance in Man:
www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

III. KIT COMPONENTS

See list of all kit components on page I.

 DNAT contains 1.6% NaOH (R 36/38).

Amplification Mix, Taq Dilution Buffer, Conjugate Solution, Wash Solution B contain 0.05% NaN_3.
Conjugate Solution contains streptavidin-alkaline phosphatase. Color Developer contains nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP).

Store all reagents at 2-8°C when not in use !

IV. MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to standard molecular biology laboratory equipment, the following is needed:

- Adjustable microcentrifuge capable of 3,000-12,000 rpm (approx. 1,000-12,000 x g)
- Incubator (e.g. heating block, water bath) capable of 56°C and 98°C (± 2°C)
- Vortex mixer
- Thermocycler (e.g. Perkin Elmer GeneAmp PCR System) and suitable thin-walled plastic reaction tubes/strips
- Waterbath with shaking platform (approx. 50 rpm) and adjustable temperature (45°C ± 0.5°C)
- Vacuum aspiration apparatus
- Shaker (rocker or orbital shaker, approx. 50 rpm)
- *Optional: agarose gel electrophoresis equipment (for control of amplification products)*
- Taq DNA Polymerase (must be licensed for use in PCR!).

V. ASSAY PROCEDURE

1. DNA Isolation

Use fresh or frozen blood with EDTA or citrate anticoagulant; avoid blood containing heparin.
Do not store blood for more than 3 days at ambient temperature or more than 1 week at 2-8°C before use. Blood which has been kept frozen for more than one year, or gone through more than three freeze-thaw cycles is unsuitable to be used in this procedure.
Bring blood samples to room temperature. Mix well by carefully inverting blood collection tubes several times. Repeat mixing each time before withdrawing an aliquot of blood.
Allow Lysis Solution and GEN^{X}TRACT Resin to reach room temperature.

- Pipette **100 µl blood sample** into a 1.5 ml microtube with screw cap.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Let stand for **15 min.** at room temperature.
- Centrifuge for **5 min.** at **3,000 rpm** (approx. 1,000 x g) in a microcentrifuge.
- Remove and discard the upper (top) 1 ml of supernatant.
- Add **1 ml Lysis Solution**, close tube and mix by inverting several times.
- Centrifuge for **5 min.** at **12,000 rpm** (approx. 12,000 x g) in a microcentrifuge.
- Remove and discard the supernatant except for approx. 50 µl of a visible, soft pellet.
- Resuspend GEN^{X}TRACT Resin by swirling the bottle thoroughly.
- Add **200 µl GEN^{X}TRACT Resin** to the pellet. Close tube and vortex for 10 sec.
  ⚠ *GEN^{X}TRACT Resin sediments quickly. Repeat resuspension each time immediately before removing another aliquot.*
- Incubate for **20 min.** at **56°C**. Vortex for 10 sec.
- Incubate for **10 min.** at **98°C**. Vortex for 10 sec.
- Centrifuge for **5 min.** at **12,000 rpm** in a microcentrifuge. Cool on ice.

The resulting supernatant contains DNA template suitable for immediate use in PCR.
For further storage, the supernatant should be transferred into a fresh tube and kept refrigerated (2-8°C; up to one week) or frozen at -20°C.

2. *In Vitro* Amplification (PCR)

Keep all PCR reagents and DNA templates refrigerated throughout.
Perform all steps until start of the thermal cycling program on ice (0-4°C).

- Prepare a fresh working dilution (0.2 U/µl) of **Taq DNA polymerase** in **Taq Dilution Buffer** (transparent cap).
- Prepare one reaction tube for each sample to be amplified. Place tubes on ice.
- For each sample prepare a final PCR reaction mix on ice:
  15 µl Amplification Mix (yellow cap)
  5 µl diluted Taq DNA polymerase (1U)
  5 µl DNA template
  If DNA templates not prepared by the kit isolation protocol (chapter V/1) are used, a DNA concentration range of 5-40 µg/ml (= 25-200 ng DNA per reaction) is recommended.

- Cap tubes tightly. Preheat the thermocycler to 94°C.
- Insert reaction tubes and run the following thermocycling program:

pre-PCR:	94°C/2 min.
thermocycling:	94°C/15 sec. - 58°C/30 sec. - 72°C/30 sec. (35 cycles)
final extension:	72°C/3 min.

Store amplification products on ice or at 2-8°C for further use.

Optional: Analyze amplification products by gel electrophoresis (e.g. 3% agarose gel).
Fragment lengths: 81, 175, 239/207, 347 bp.

3. Hybridization (45°C; shaking waterbath)

Adjust the water level of the waterbath to approx. ½ of the height of the Typing Tray.
Heat the waterbath to exactly 45°C (± 0.5°C). Check water temperature with a calibrated thermometer.
Prewarm Hybridization Buffer and Wash Solution A to 45°C. (Take care that all precipitates formed at 2-8°C become completely dissolved.)
Allow Teststrips, DNAT, Conjugate Solution, Wash Solution B and Color Developer to reach room temperature. Prepare Typing Tray(s).
Remove one Teststrip for each sample using clean tweezers. (Touch Teststrips with gloves only!)
Label Teststrips outside of the marker lines with a pencil. (No ballpoint pens, markers, etc.)

- Pipette **10 µl DNAT** (blue cap) into the lower corner of each lane to be used in the Typing Trays (one lane per sample).
- Add **10 µl amplification product** into the corresponding drop of DNAT.
  Mix thoroughly with a pipette. *(The solution will remain blue.)*
- Let stand for **5 min.** at room temperature.
- Add **1 ml Hybridization Buffer** (prewarmed to 45°C) into each lane.
  Gently agitate tray. *(The blue color will disappear.)*
- Insert **Teststrips** with marked side up (lines visible!) into the respective lanes.
  Submerge completely.
- Incubate for **30 min.** at **45°C** on the shaking platform of the waterbath.
  Set moderate shaking frequency (approx. 50 rpm) to avoid spilling.
  Keep the cover of the waterbath closed to avoid variations in temperature.
- At the end of incubation remove hybridization solutions by vacuum aspiration.
  Proceed immediately. Do not allow Teststrips to run dry during the entire procedure.

4. Stringent Wash (45°C; shaking waterbath)

- Add **1 ml Wash Solution A** (prewarmed to 45°C). Rinse briefly (10 sec.).
  Remove liquids by vacuum aspiration.
- Add **1 ml Wash Solution A** (45°C).
- Incubate for **15 min.** at **45°C** in the shaking waterbath.
  Remove liquids by vacuum aspiration.
- Add **1 ml Wash Solution A** (45°C).
- Incubate for **15 min.** at **45°C** in the shaking waterbath.
  Remove liquids by vacuum aspiration.

5. Color development (room temperature)

- Add **1 ml Conjugate Solution**.
- Incubate for **15 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration.
- Add **1 ml Wash Solution B**. Rinse briefly (10 sec.).
  Remove liquids by vacuum aspiration.
- Add **1 ml Wash Solution B**.
- Incubate for **5 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration.
- Add **1 ml Wash Solution B**.
- Incubate for **5 min.** at **room temperature** on a rocker or orbital shaker.
  Remove liquids by vacuum aspiration.
- Add **1 ml Color Developer**.
- Incubate for **15 min.** at **room temperature** in the dark on a rocker or orbital shaker
  A purple staining will appear upon positive reaction.
- Wash Teststrips several times with distilled water.
  Let strips dry in the dark on absorbent paper.
  Do not expose Teststrips to intense light after Color Development.

VI. INTERPRETATION OF RESULTS

The genotype of a sample is determined using the enclosed Collector™ sheet.
Place the processed Teststrip into one of the designated fields, align it to the schematic drawing using the red marker line (top) and the green marker line (bottom), and fix it with adhesive tape.

A positive reaction of the uppermost Control line indicates the correct function of Conjugate Solution and Color Developer. This line should always stain positive.

For each polymorphic position, one of the following staining patterns should be obtained:
Note: Staining intensities of positive lines may vary. This is of no significance for the result.

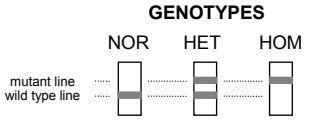


Fig. 2a

	wild type line	mutant line	genotype
NOR	**positive**	negative	normal
HET	**positive**	**positive**	heterozygous
HOM	negative	**positive**	homozygous mutant

The CYP2D6 alleles *1 (wild type), *3, *4, *6, and the resulting homozygous and heterozygous genotypes (*1/*1, *1/*3, etc.) are determined by probes for mutations 2637delA, 1934 G>A and 1795delT, respectively (Fig. 2b).

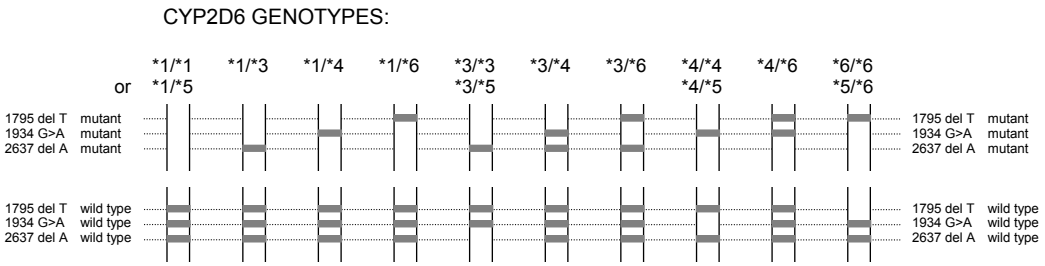


Fig. 2b

The PGX-HIV StripAssay ™ does not differentiate homozygosity for each of these alleles from compound heterozygosity with allele *5, which represents a deletion of the entire CYP2D6 gene (e.g. *3/*3 versus *3/*5). With respect to drug-metabolizing phenotype (extensive versus poor) a discrimination is not necessary, since both respective genotypes will fall into the same category. In case of homozygous *5 allele (*5/*5), no CYP2D6 PCR products will be generated, and all corresponding wild type and mutant signals will be missing (see example J, page III).
In all these cases the CYP2D6 gene deletion (*5 allele) is only indirectly demonstrated; however, it can be further confirmed by suitable long-range PCR approaches (e.g. Steen et al. 1995, Pharmacogenomics 5, 215-23).

phenotype	**CYP2D6 genotype**
extensive metabolizer (EM)	*1/*1, *1/*3, *1/*4, *1/*5, *1/*6
poor metabolizer (PM)	*3/*3, *3/*4, *3/*5, *3/*6, *4/*4, *4/*5, *4/*6, *5/*5, *5/*6, *6/*6

See examples of StripAssay results on page III (Fig. 3).

A detailed Technical Handbook for StripAssays is available for download at: www.viennalab.com. Advise on throubleshooting may be obtained from there, as well as by contacting ViennaLab through the local distributor or directly at the address provided on page I.

VII. QUALITY CONSIDERATIONS

- A thorough understanding of the procedure outlined here, and precise laboratory equipment and techniques are required to obtain reliable results. Use of the StripAssay for human *in vitro* diagnostics needs to be limited to appropriately trained personnel.
- Do not use StripAssay components beyond the expiration date printed on the outside of the kit box. Do not mix reagents from different lots.
- Avoid microbial contamination and cross-contamination of reagents or samples by using sterile disposable pipette tips throughout. Do not interchange bottle caps.
- The Control line immobilized on each Teststrip allows a performance control of the chromogenic detection system. To monitor and validate the specificity of the hybridization and washing steps, control DNAs of known genotype should be included into each individual experiment.

VIII. SAFETY

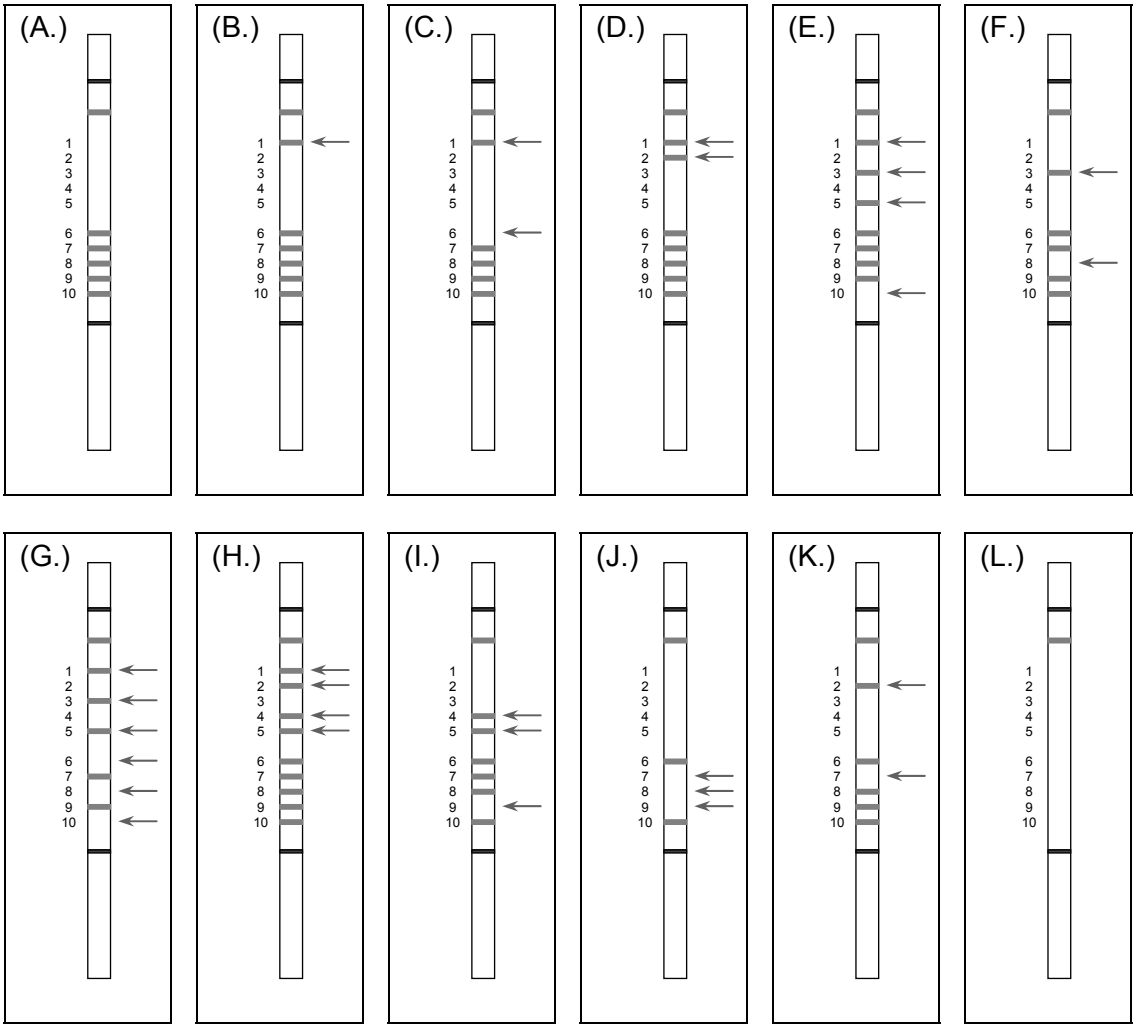
- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and disposable gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with clinical specimens in a biohazard waste container.
- Avoid contact of DNAT with skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. If spilled, dilute with water before wiping dry.
- Adhere to all local and federal safety and environmental regulations which may apply.

IX. PCR LICENSE

The PCR process is covered by patents owned by F. Hoffmann-La Roche Ltd. and Roche Molecular Systems Inc. Use of the PCR process requires a license.

PGX-HIV StripAssay ™ (4-710) does not convey a license to use the PCR process and is intended for use under existing PCR license agreements only.

Fig. 3: Examples of test results



	MDR1	CYP2D6	CCR5
(A.)	normal	*1/*1 or *1/*5	normal
(B.)	heterozygous	*1/*1 or *1/*5	normal
(C.)	homozygous	*1/*1 or *1/*5	normal
(D.)	heterozygous	*1/*6	normal
(E.)	heterozygous	*1/*4	homozygous
(F.)	normal	*4/*4 or *4/*5	normal
(G.)	homozygous	*4/*4 or *4/*5	homozygous
(H.)	heterozygous	*3/*6	heterozygous
(I.)	normal	*3/*3 or *3/*5	heterozygous
(J.)	normal	*5/*5 or partial PCR failure	normal
(K.)	normal	*5/*6 or *6/*6	normal
(L.)	negative control or PCR failure		

REF

Σ

4-710	PGX-HIV StripAssay™	20 tests
4-710-AM	Amplification Mix for PGX-HIV StripAssay™	500 µl
6-080	Typing Trays	5
2-014	GEN^{X}TRACT Blood DNA Extraction System	100 extractions

Distributed by:

Manufactured by:

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