Apo E StripAssay ®

REF 4-280

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	⚠ Warning
6.	**Typing Trays**	3	
7.	**Teststrips**	20	
8.	**Hybridization Buffer** *(white cap)*	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	⚠ Warning
13.	**Instructions For Use**	1	
14.	**Collector™ Sheet**	1	

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Ref.Seq. NM_000041.2

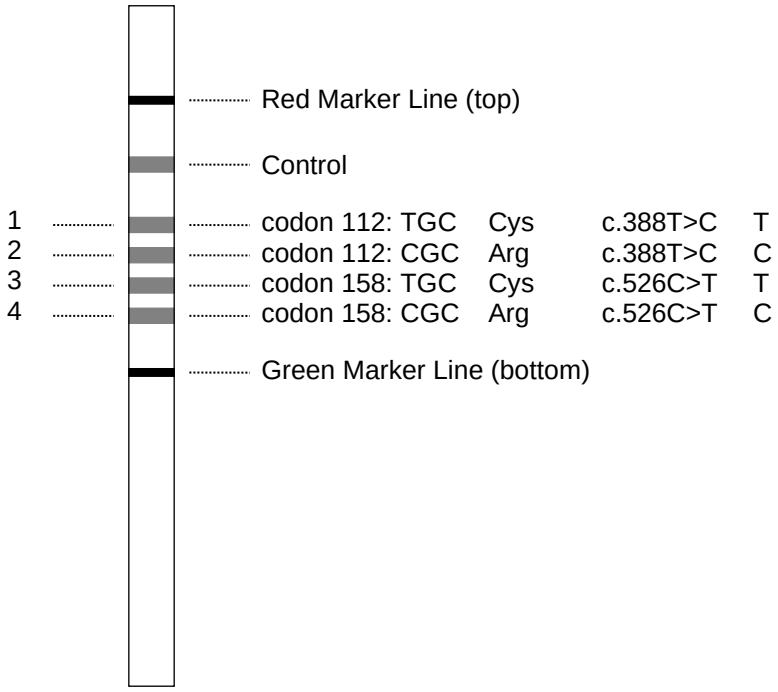

Fig. 1: Teststrip Design
Note: Teststrip is not drawn in real size and must not be used for interpretation of results!

Instructions for use

I. INTENDED USE

Assay for the identification of apolipoprotein (apo) E isoforms E2, E3 and E4 based on polymerase chain reaction (PCR) and reverse-hybridization. *For human in vitro diagnostics.*

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 allows the discrimination between six possible heterozygous or homozygous apo E genotypes: E2/2, E2/3, E2/4, E3/3, E3/4, E4/4.
Further genetic information is available at OMIM Online Mendelian Inheritance in Man: www.ncbi.nlm.nih.gov/omim

III. KIT COMPONENTS

See list of all kit components on page I.

Warning: DNAT contains 1.6% NaOH.
H315: Causes skin irritation
H319: Causes serious eye irritation
P280: Wear protective gloves/protective clothing/eye protection/face protection
P337 + P313: If eye irritation persists: Get medical advice/attention

Warning: Color Developer contains ≤0.4% maleic acid.
H317: May cause an allergic skin reaction
P280: Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352: If on skin: wash with plenty of water
P333 + P313: If skin irritation or rash occurs: get medical advice /attention

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 (1,000-12,000 x g)
- Incubator (e.g. heating block, water bath) capable of 56°C and 98°C (± 2°C)
- Thermocycler and suitable thin-walled plastic reaction tubes/strips
- Taq DNA polymerase (ViennaLab REF TAQ-500 / TAQ-2500)
- Waterbath with shaking platform and adjustable temperature (45°C ± 1°C)
- Vacuum aspiration apparatus
- Shaker (rocker or orbital shaker)
- *Optional: agarose gel electrophoresis equipment (for control of amplification products)*

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 2-10 ng/µl (= 10-50 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: 232 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 (± 1°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 the three apo E isoforms E2, E3 and E4 the following staining patterns are obtained:
Note: Staining intensities of positive lines may vary. This is of no significance for the result.

E2 (112: Cys, 158: Cys)	lines (1) + (3)
E3 (112: Cys, 158: Arg)	lines (1) + (4)
E4 (112: Arg, 158: Arg)	lines (2) + (4)

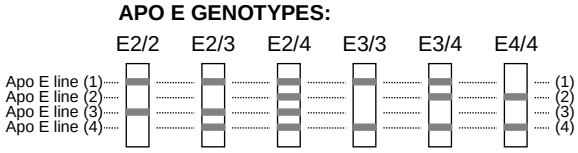

Fig. 2

The six possible homozygous and heterozygous apo E genotypes (E2/2, E3/3, E4/4, E2/3, E2/4, E3/4) will result in a combination of the respective individual isoforms (Fig. 2).

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

Advise on troubleshooting may be obtained by contacting ViennaLab through the local distributor or directly at techhelp@viennalab.com.

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.