ViennaLab®

StripAssay®
Technology & Procedure

ViennaLab StripAssays®

Human diagnostic assays for genotyping mutations and polymorphisms (SNPs, deletions, insertions) based on **polymerase chain reaction (PCR)** and reverse-hybridzation to allele-specific oligonucleotide probes immobilized on **teststrips**.

StripAssay® components

SIMPLE

ECONOMICAL

RELIABLE

RAPID

EFFICIENT

CONVENIENT

Ready-to-use Amplification Mixes, Teststrips, processing reagents and incubation trays.

StripAssay® components

Materials required but not supplied*):

- 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
- waterbath with shaking platform and adjustable temperature (45°C ± 1°C)
  alternatively: thermoshaker with heated lid (selected models only!) plus simple non-shaking waterbath
- vacuum aspiration apparatus
- shaker (rocker or orbital shaker)
- for most StripAssays®: Taq DNA polymerase (avoid hot-start enzymes!)
- for DNA from external kits/protocols: spectrophotometer (UV 260 nm)
  for DNA extracted from FFPE tissue: fluorometer (e.g. Qubit system)
- optional: agarose gel electrophoresis equipment
  (for control of amplification products)

*) *In addition to standard molecular biology laboratory equipment*

StripAssay® procedure

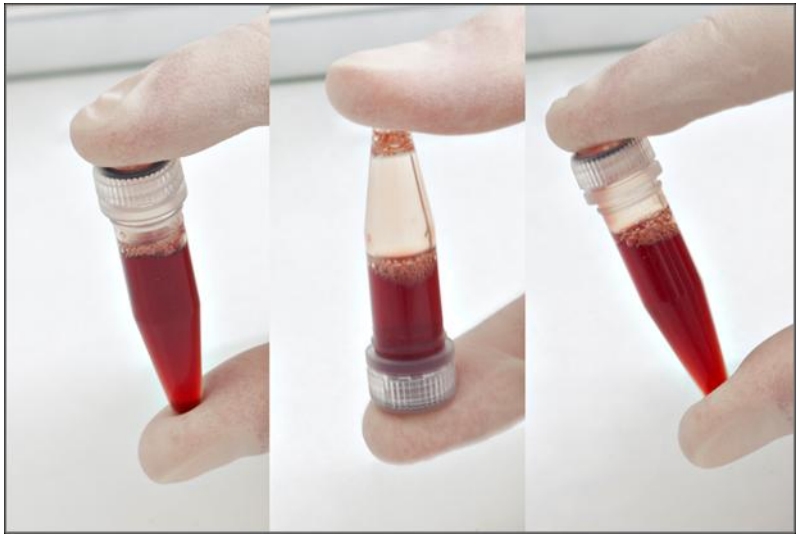
1. **DNA Isolation**
2. ***In Vitro* Amplification (PCR)**
3. **Hybridization**
4. **Stringent Washes**
5. **Color Development**
6. **Interpretation**

1. DNA Isolation from blood (GEN^{X}TRACT)

- Use **fresh** or **frozen blood** (anticoagulant: EDTA; avoid citrate or heparin)
  max. **3 days** at **ambient temperature**
  max. **1 week** at **2-8ºC**
  max. **1 year frozen**
- **Avoid** repeated **freeze-thaw** cycles
- Bring blood samples to **room temperature**
- Before withdrawing an aliquot, **mix well** by inverting blood collection tubes several times
- 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 micro-centrifuge.

Remove and discard the upper (top) 1 ml of supernatant.

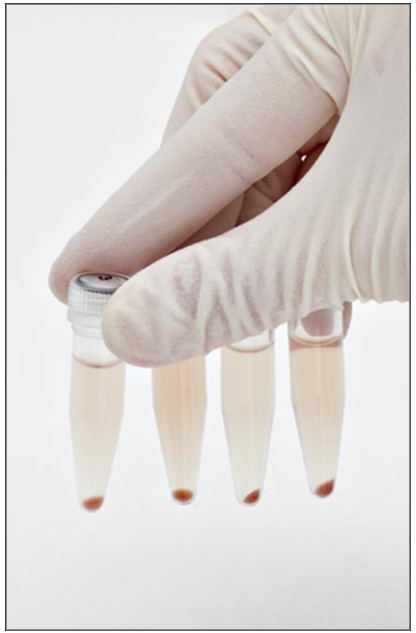
Leave pellet undisturbed !

Pellet should be completely resuspended !

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.

GEN^{X}TRACT Resin sediments quickly. Repeat resuspension each time immediately before removing another aliquot !

Add **200 µl GEN^{X}TRACT Resin** to the pellet.

Close tube and vortex for 10 sec.

Incubate for 20 min at 56ºC.

Vortex for 10 sec.

Incubate for **10 min** at **98ºC**.

Vortex for 10 sec.

Centrifuge for **5 min** at **12,000 rpm** in a micro-centrifuge. 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.

2. *In Vitro* Amplification (PCR)

- Use **10-50 ng** DNA per reaction (**2-10 ng/µl**), if DNA templates are not prepared by the GEN^{X}TRACT protocol
  (DNA from FFPE tissue: use **1-10 ng/µl** quantified by fluorometry)
- Keep all **PCR reagents** and **DNA templates refrigerated**
- Prepare one reaction tube for each sample and place tubes on ice
- Perform all steps **on ice** (0-4ºC) until start of the thermocycler
- **Preheat** thermocycler to starting temperature (usually 94ºC)

Prepare a fresh working dilution (usually 0.2 U/µl) of **Taq DNA polymerase** in **Taq Dilution Buffer** (transparent cap).

For each sample prepare a final PCR reaction mix on ice:

15 µl Amplification Mix (yellow or green or white cap)

+ 5 µl diluted Taq DNA polymerase (usually 1U)

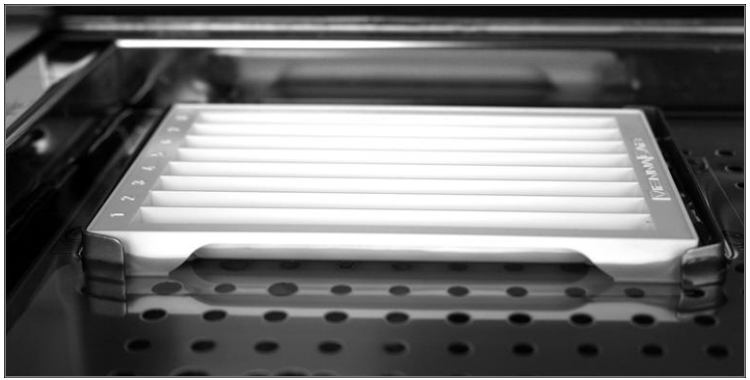
+ 5 µl DNA template

Cap tubes tightly.
Insert reaction tubes into the preheated thermocycler and run the appropriate program.

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

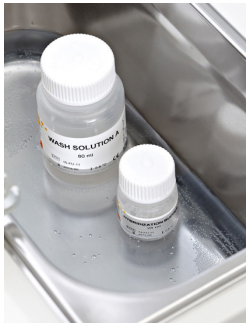
3. Hybridization (at 45°C)

- Use a **shaking waterbath**
- Adjust water level to approx. ½ **of the height of the Typing Tray**
- Set temperature to exactly **45ºC**
- Check water temperature with a fully inserted calibrated thermometer



..... or alternatively:

Use **thermoshaker with heated lid** (for incubations) plus a simple waterbath (for preheating reagents).



Set temperature to exactly 45°C.

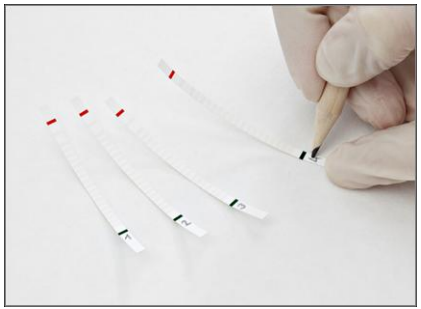
*Do **not** use **air-based** hybridization devices because of insufficient heat transmission (e.g. hybridization oven).*

Prewarm Hybridization Buffer and Wash Solution A to 45ºC.

Take care that all precipitates formed at 2-8ºC become completely dissolved after prewarming.

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, ink 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 45ºC) into each lane. Gently agitate tray.

The blue color will disappear !

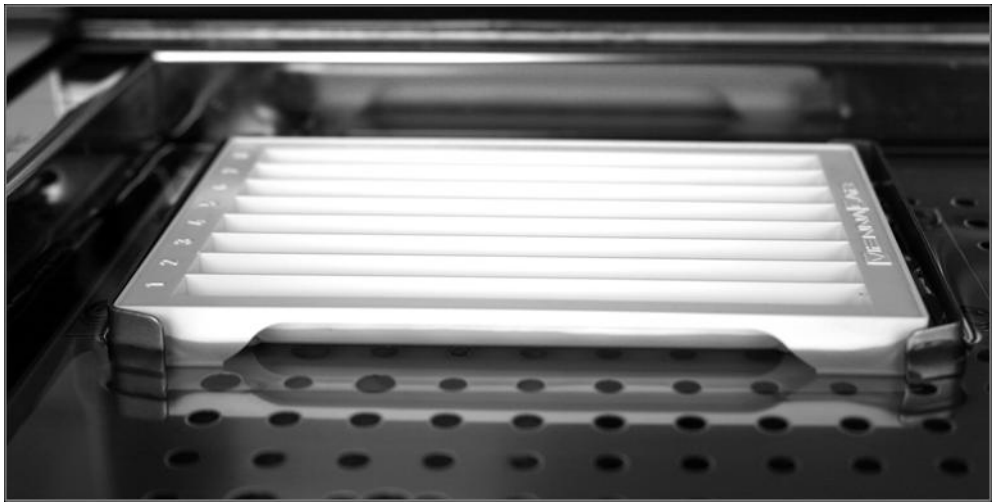
Insert **Teststrips** with marked side up (lines visible!) into the respective lanes.

Submerge Teststrips completely.

*In order to avoid cross-contamination, do **not** dip tweezers into solution.*

Incubate for **30 min** at **45ºC** on the shaking platform of the waterbath or thermoshaker.

Set moderate shaking frequency to avoid spilling. Keep the cover of the waterbath or thermoshaker 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 Washes (at 45°C)

One **rinsing** step:
Add **1 ml Wash Solution A** prewarmed to 45ºC. Rinse briefly (10 sec). Remove liquids by vacuum aspiration.

Two **washing** steps:
Add **1 ml Wash Solution A** (45ºC).
Incubate for **15 min** at **45ºC** on the shaking platform of the waterbath or thermoshaker.
Remove liquids by vacuum aspiration.

Repeat 1 x

5. Color Development (at 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.

One **rinsing** step:
Add **1 ml Wash Solution B.** Rinse briefly (10 sec). Remove liquids by vacuum aspiration.

Two **washing** steps:
Add **1 ml Wash Solution B**. Incubate for 5 min at room temperature on a rocker or orbital shaker. Remove liquids by vacuum aspiration.
Repeat 1 x

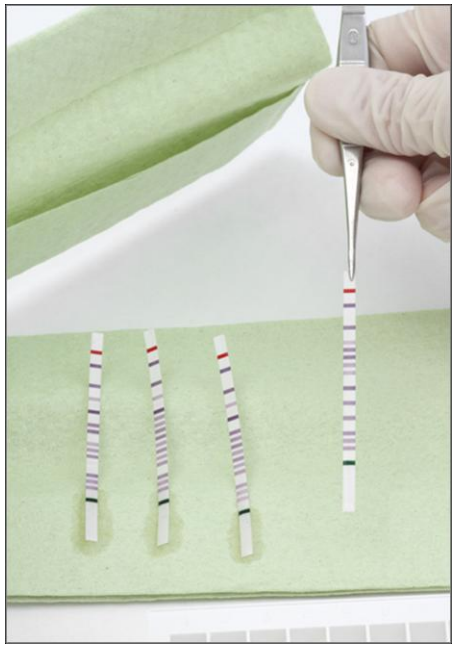
Add **1 ml Color Developer**.
Incubate for **15 min** at **room temperature** in the dark on a rocker or orbital shaker.

A purple staining will appear upon positive reaction.

Wash Teststrips several times with distilled water.

Do not expose Teststrips to intense light after Color Development.

Let strips dry <u>in the dark</u> on absorbent paper.



Optional: **Automated processing of teststrips**

Three steps on one instrument: Hybridization + Stringent Washes + Color Development
fully automated under controlled temperature

for 48 teststrips:
ProfiBlot T48 or **BeeBlot**

for 20/30 teststrips:
Dynablot Heat or **Autoblot 3000H** or **B20**

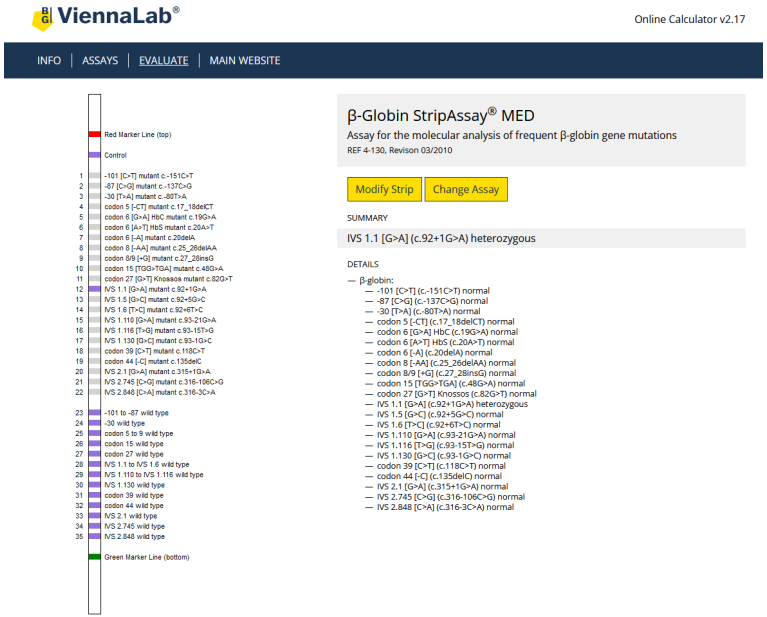
6. Interpretation

Place processed and **dried** Teststrips into the designated fields of the Collector™ sheet.

Align them to the schematic drawing using the red marker line (top) and the green line (bottom) and fix them with adhesive tape.

Optional: **Automated recording, interpreting and electronic archiving of results**

Scan Collector™ sheet and use **StripAssay® Evaluator** software for automated interpretation of band patterns.



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