

StripAssay® Troubleshooting Guide



Problem	Possible cause	Comments and suggested solutions
Weak or missing WT and MUT signals, upper control line present	<u>Inefficient or failed amplification</u>	
	Fragmented DNA	Repeat DNA extraction
	Input DNA amount out of - usually below - the specified working range of the assay (10 to 100 ng in total per reaction)	Check DNA concentration; if concentration is too low repeat DNA extraction
	Amplification failure due to presence of PCR inhibiting substances in DNA preparation	Re-purify DNA or dilute DNA with PCR grade water prior to PCR (use a concentration of at least 2 ng/μl)
	Incorrect Taq DNA Polymerase dilution (inappropriate dilution or dilution buffer or not freshly prepared)	Prepare fresh and correct Taq DNA Polymerase dilution in Taq Dilution Buffer supplied with the kit
	Defective, low-quality or improper (e.g. Hotstart) Taq DNA Polymerase	Use Taq DNA Polymerase provided by ViennaLab or other suitable Taq polymerase
	Incorrect volumes of Amplification Mix, diluted Taq DNA Polymerase or DNA used in the assay	Use calibrated pipettes and carefully control the dispensed reagent volumes (see Instructions For Use)
	Incorrect PCR program or unsuitable PCR tubes used	Verify PCR program, use thin-wall PCR tubes
	<u>Low hybridization efficiency</u>	
	Hybridization temperature too high (> 45°C)	Adjust temperature of hybridization device to exactly 45°C (+/- 0.5°C) and check temperature with calibrated external thermometer; fully submerge mercury thermometer!
Precipitates in Hybridization Buffer and Wash Solution A	Prewarm buffers to 45°C (+/- 0.5°C) and mix well to dissolve precipitates prior to use	
Incorrect incubation time during hybridization steps	Do not prolong or shorten incubation time. Exactly follow the Instructions For Use	
All signals weak or missing, including upper control line	Systematic error in the protocol	Repeat assay according to Instructions For Use
	Kit reagents expired, deteriorated or stored improperly	Repeat assay with fresh reagents

Problem	Possible cause	Comments and suggested solutions
<p>Drop-out of WT and MUT signals for one marker</p> <p>Other markers show normal staining.</p>	<p>Partial drop out of multiplex PCR due to (1) PCR setup at room temperature and / or (2) thermoblock of PCR cycler not preheated</p>	<p>Control PCR with gel electrophoresis. If amplicons are missing repeat PCR: Use cold reagents and set up PCR on ice or cooling block; preheat thermoblock to specified temperature, only then insert PCR tubes into PCR cycler</p>
	<p>Special genetic situation, e.g. rare SNP or deletion present in the primer and / or probe binding site</p>	<p>Sequencing analysis of the sample is indicated</p>
<p>High background or presumptive false positive results</p>	<p>Input DNA amount above the specified working range of the assay (10 to 100 ng in total per reaction)</p>	<p>Check DNA concentration using fluorometric DNA measurement (e.g. Qubit)*, adjust to 2 to 20 ng/μl with PCR grade water</p>
	<p>Incorrect Taq DNA Polymerase dilution (inappropriate dilution or dilution buffer or not freshly prepared)</p>	<p>Prepare fresh and correct Taq DNA Polymerase dilution in Taq Dilution Buffer supplied with the kit</p>
	<p>PCR setup at room temperature</p>	<p>Use cold reagents and set up PCR on ice or cooling block</p>
	<p>Thermoblock of PCR cycler not preheated</p>	<p>Preheat thermoblock to specified temperature, only then insert PCR tubes into PCR cycler. Important note: oncology StripAssay® (BRAF, EGFR, KRAS, NRAS) PCR starts with 37°C</p>
	<p>Precipitates in Hybridization Buffer and Wash Solution A</p>	<p>Prewarm buffers to 45°C (+/- 0.5°C) and mix well to dissolve precipitates prior to use</p>
	<p>Mix up of Wash Solution A and Wash Solution B</p>	<p>Use correct reagents according to the Instructions For Use</p>
	<p>Hybridization temperature too low (< 45°C)</p>	<p>Adjust temperature of hybridization device to exactly 45°C (+/- 0.5°C) and check temperature with calibrated external thermometer; fully submerge sensor of thermometer!</p>
	<p>Insufficient heat transmission due to too low water level in the waterbath or incubation with open lid</p>	<p>Adjust water level to ½ of the height of the incubation tray; keep lid of waterbath closed to avoid temperature variations and evaporation</p>
	<p>Use of inappropriate hybridization device (e.g. air-based incubators)</p>	<p>Use shaking waterbath or recommended thermoshaker with heated lid (Biosan PST-60HL, Biosan PST-60HL-4)</p>
<p>Incorrect incubation time during hybridization steps</p>	<p>Do not prolong or shorten incubation time. Exactly follow the Instructions For Use</p>	

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	Untimely analysis of still wet Teststrips	Let Teststrips dry completely (avoid exposure to light) before interpretation of results
Positive bands in no-template control (NTC)	DNA contamination of the NTC sample itself and/or PCR reagents and/or equipment	Carry out DNA extraction and PCR setup using filter-tips along with dedicated pipettes. Clean workbench and equipment; repeat assay with fresh reagents
	Cross-contamination of neighbouring lanes in the tray during hybridization	Apply moderate shaking frequency to avoid lane-to-lane spilling
Unstained areas along band pattern of the Teststrips	Teststrips placed face down into tray	Insert Teststrips with glossy side towards the bottom
	Incorrect volumes of detection reagents used	Use calibrated pipettes and carefully control the dispensed reagent volumes (see Instructions For Use)
High background staining of entire Teststrip(s)	Color development was not carried out shaking and/or reaction was exposed to direct light	Carry out all incubation steps on a shaking platform, avoiding exposure to light during color development
	Color development was carried out at high (>25°C) ambient temperature	Reduce ambient temperature below 25°C
	Color Developer was insufficiently rinsed off	Rinse Teststrips several times with distilled water
Applies specifically to α-Globin StripAssay® (4-160):		
PCR Control A and B missing, WT bands present	PCR products were hybridized to inappropriate Teststrip (Mix A1 + A2 to Teststrip B and Mix B to Teststrip A)	Repeat hybridization and add amplicons to corresponding Teststrip
PCR Control A missing	PCR products of Mix A2 were not hybridized to Teststrip A	Repeat hybridization and add amplicons to corresponding Teststrip
Difficulties to amplify -3.7del and anti-3.7 mutations	Input DNA amount too high and/or contamination with PCR inhibitors (particularly applies for magnetic-particle based extraction systems)	Dilute DNA with PCR grade water to 2 to 20 ng/μl prior to PCR

Problem	Possible cause	Comments and suggested solutions
Applies specifically to BRAF (5-560, 5-570), KRAS (5-580, 5-590, 5-680), NRAS (5-620) and EGFR (5-630)-StripAssays®:		
PCR Positive Control missing or very weak	Poor quality input DNA (degraded or containing inhibitors)	Repeat DNA extraction or re-purify DNA
	Input DNA amount below the specified working range of the assay (5 to 50 ng in total per reaction)	Check DNA concentration, using fluorometric DNA measurement (e.g. Qubit)*; if concentration is too low repeat DNA extraction. Important note: DNA extracted from formalin-fixed and paraffin embedded tissue (FFPE) cannot be quantified correctly using UV-VIS based methods (e.g. Nanodrop)
	Inhibition of Taq DNA Polymerase	Dilute DNA with PCR grade water
	Highly compromised DNA quality due to formalin fixation	Repeat DNA extraction
Faint background and / or PCR Negative Control signal present	Impaired blocking and resulting amplification of wild-type allele due to excessive DNA input amount	Check DNA concentration using fluorometric DNA measurement (e.g. Qubit)*, adjust to 1 to 10 ng/μl with PCR grade water. Important note: DNA extracted from formalin-fixed and paraffin embedded tissue (FFPE) cannot be quantified correctly using UV-VIS based methods (e.g. Nanodrop)
	Low quality of FFPE DNA contaminated with substances interfering with specificity of primers and wild-type-blocking	Avoid overloading FFPE DNA extraction columns; a diligent processing of FFPE material according to the manufacturer's protocol for DNA extraction ensures minimal carry-over of contaminants
	DNA samples from stained tissue (e.g. May Grünwald Giemsa)	Use unstained tissue material
	Formalin over-fixation of tissue samples and/or use of old or unbuffered formalin solution for fixation	Use fresh and neutral buffered formalin, avoid excessive fixation
	Ramp rate of thermocycler too low (< 1°C/sec). Excessive amount of template input may further increase background	Change ramp rate in thermocycler settings or use suitable instrument

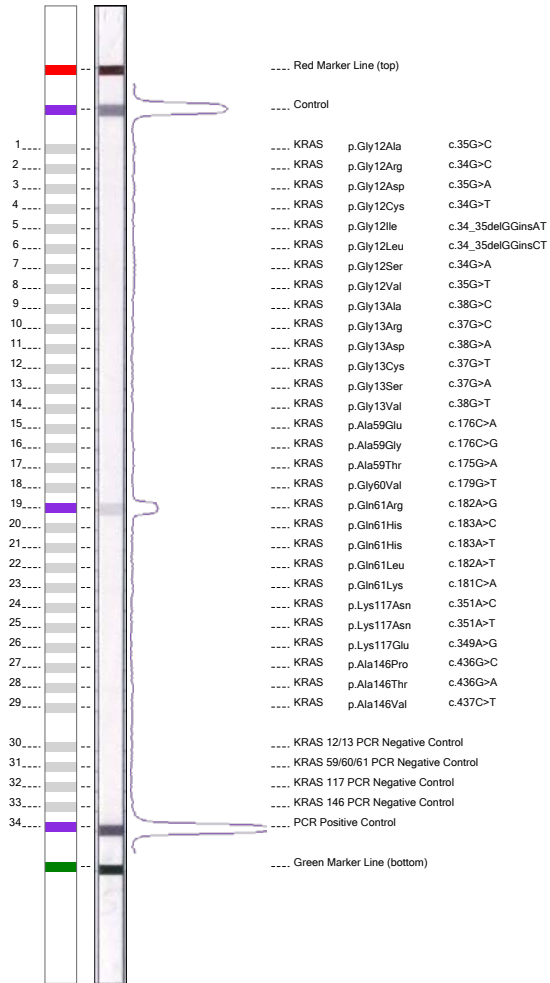
Problem	Possible cause	Comments and suggested solutions
Unclear mutation signals	Weak signals may be the result of the presence of a low frequency mutation	Repeat PCR and hybridization. A mutation with low frequency present in the sample is reproducible. Within a range, the intensity of a mutation signal will change with the template amount used in PCR
	Multiple faint signals for variable mutations caused by insufficient DNA quality or deviation from the protocol	Consider purification of the original DNA. Check the DNA concentration, repeat PCR and hybridization. The faint pattern of formalin-induced artefacts is usually not reproducible
<p>* Please verify the use of the correct Qubit kit: dsDNA BR (Range 2 to 1000ng) or dsDNA HS (Range 0,2 to 100ng) for DNA quantification</p>		

General considerations:

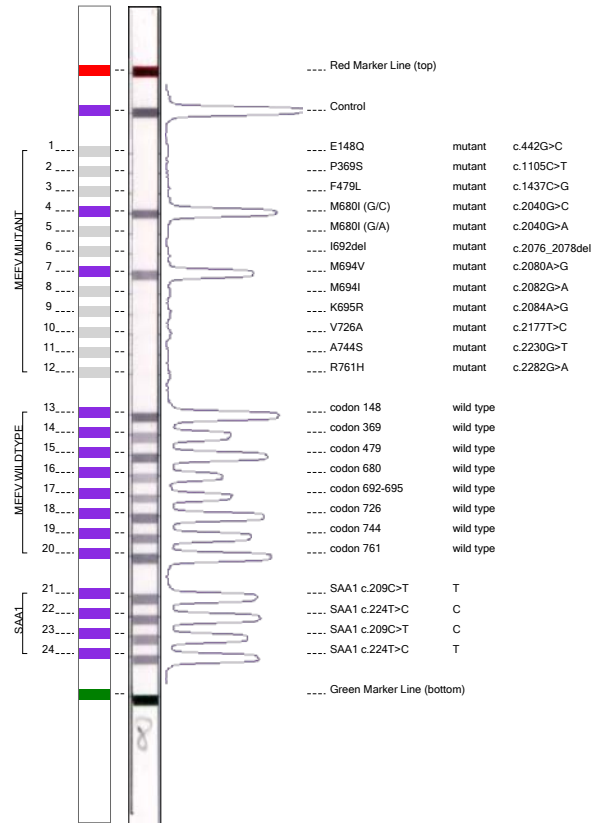
1. Keep pre- and post-PCR workspace separated
2. Use suitable equipment (shaking waterbath or recommended thermoshaker)
3. Follow exactly the Instructions For Use

Examples of StripAssay® results:

5-680 KRAS XL StripAssay®



4-390 FMF-SAA1 StripAssay®



4-360 CVD StripAssay® T

