Catalog #: T0500S

ACTN3 Sports Gene Test Kit

Research use only

01 INTRODUCTION

The human ACTN3 gene encodes α-actinin-3, an actin binding protein that plays an important role in muscle structure and metabolism. The ACTN3 R577X (rs1815739) polymorphism is a functional polymorphism caused by a common genetic single nucleotide polymorphism (SNP) at codon 577 of the ACTN3 gene and results in the replacement of an arginine (R) with a stop codon (X). The R allele is a normal functional version of the gene, whereas the X allele contains a sequence change that completely stops production of functional α-actinin-3 protein. The ACTN3 R577X was found to be associated with power athletic performance, especially among male and female track and field athletes. Individuals who are homozygous for the wild type allele (RR) or heterozygous (RX) for the two alleles make the ACTN3 protein. Individuals who are homozygous for the mutant (XX) allele do not produce the ACTN3 protein. The frequency of RR (the genotype for speed and power) is 0.25 in Asians, 0.36 in European whites, 0.60 in African-Americans, and 0.81 in African Bantu. According to a study by Mills et al., 2001, there is one RR for every XX among Asians, nearly two RRs for every XX among whites, and more than four RRs for every XX among African-Americans.

The ACTN3 Sports Gene Test Kit uses Real-Time PCR followed by melt curve analysis (MCA) to determine the ACTN3 genotype (RR, RX, or XX). The kit contains all reagents required to make a crude extract from buccal cells. The crude cell extract contains sample DNA to amplify the target gene. Also provided are specific primers and Chai Green qPCR Master Mix for amplifying the R577X locus followed by MCA. The Chai Green qPCR Master Mix is a mix for dye-based Real-Time PCR, melt curve analysis, and High Resolution Melt (HRM). Chai Green is a DNA binding dye that will fluoresce when intercalated between double-stranded DNA (dsDNA). This is essential as fluorescence signal will rapidly decrease as target dsDNA is slowly denatured in the melt curve protocol. The shape and peaks of the melt curve are used for genotyping.

02 APPLICATIONS

ACTN3 genotyping (RR, RX, or XX)

03 SAMPLE TYPE

DNA from crude extracts of human buccal cells

04 KIT CONTENTS

Kit Components	Volume/Quantity
Extraction Buffer A	25 mL
Enzyme B	Lyophilized
Reconstitution Buffer "C"	0.5 mL
ACTN WT (standard)	Lyophilized
ACTN Mutant (standard)	Lyophilized
ACTN Primers	Lyophilized
Chai Green qPCR Master Mix	1 mL
Water	5 mL
Swabs	50
1.5 mL tube	60
8-strip PCR Strip Tubes	15
8-strip PCR Strip Caps	15

05 ADDITIONAL REQUIRED MATERIALS

a. Thermocycler with capabilities for Real-Time PCR and melt curve analysis, such as the Open qPCR
b. Low speed mini centrifuge for microfuge tubes and PCR tubes
c. Vortexer
d. Tube racks for PCR tubes and microfuge tubes
e. Pipettes and pipette tips

06 STORAGE CONDITIONS AND REAGENT PREPARATION

Store all reagents at -20 ºC for long-term storage. Once the various components are reconstituted, store at -20 ºC and avoid repeated freeze-thaw cycles. Protect the Chai Green qPCR Master Mix from prolonged exposure to light. Always spin down tubes in a centrifuge to prevent contents from spilling out when opening the tube caps.

1. Pipette 0.5 mL of Reconstitution Buffer "C" into the vial containing lyophilized Enzyme B using an appropriately sized pipette. Mix well by pipetting up and down ten times or until the lyophilized crystal is fully dissolved. Cap and spin down the tube contents. Enzyme B is now reconstituted.

2. Pipette 0.5 mL of reconstituted Enzyme B to the bottle containing 25 mL of Extraction Buffer A in the bottle and gently swirl to mix. Label this the Chai Buccal Cell Extraction Solution. Store this at 4 °C (short-term) or -20 °C (long-term), avoiding repeated freeze-thaw cycles. The product is stable for two years from date of receipt when stored at -20 °C without freeze-thaw.

3. Reconstitute the ACTN3 Primer by pipetting 275 µL of water into the ACTN3 tube. Mix well by pipetting up and down ten times.

4. Reconstitute each ACTN WT and ACTN Mutant (DNA standard) by pipetting 50 µL of water into each tube and mix by pipetting up and down ten times. Keep at room temperature for an hour so that all components go into solution. Vortex for a few seconds, then spin down the tubes briefly to collect contents at the bottom of the tubes.

07 PROTOCOL

1. **Sample Preparation:** Pipette 0.5 mL (500 µL) of the Chai Buccal Cell Extraction Solution into a new, sterile 1.5 mL tube. Collect buccal cells by rolling a swab firmly on the inside of the cheek approximately 20 - 30 times. Insert the swab with the collected cells into the tube. If you want higher yields of DNA in the crude extract, you can include more cells by using two buccal swabs per 500 µL the Chai Buccal Cell Extraction Solution. Rotate the brush vigorously in the tube approximately ten times to dislodge the cells, then push the brush against the side of the tube to ensure that most of the liquid remains in the tube. Cap the tube and vortex to mix, then spin down for approximately 3 seconds to collect the tube contents at the bottom.

2. **Preparation of Crude Cell Extract:** Set the protocol in the Open qPCR instrument as shown in Figure 1 below. From the default protocol, delete all steps in Stage 2, add another step to Stage 1, and adjust the temperatures and hold times accordingly. Mix your prepared sample from step one by pipetting up and down ten times, then transfer 100 µL of the solution to a new, sterile 0.1 mL PCR tube. Insert the PCR tube containing the sample in the Open qPCR instrument.

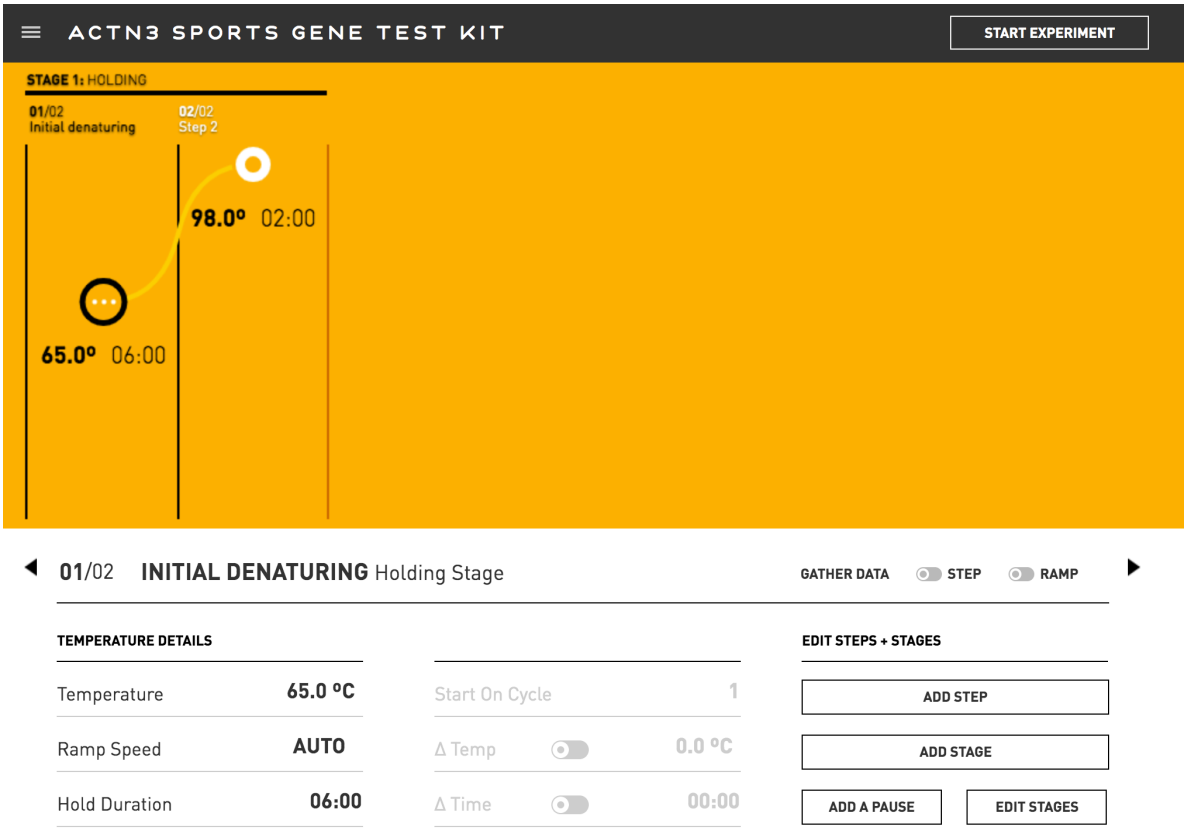

Figure 1. Open qPCR protocol for preparation of crude cell extract.

Start the experiment in the protocol window. Heating the sample at 65 ºC for six minutes activates the enzyme in your Chai Buccal Cell Extraction Solution, which will promote lysis of sample buccal cells and release the DNA into solution. Additionally, enzymes in the solution will digest interfering cellular proteins and nucleases. Heating the sample at 98 ºC for two minutes will inactivate these enzymes that would otherwise interfere with downstream applications such as PCR or qPCR. Once the protocol completes, carefully remove the PCR tube (the lid and the tube holder will be hot) from the Open qPCR and allow the contents to cool to room temperature. Spin down the tube for one minute until a pellet forms at the bottom. The pellet contains remaining cell debris, while the supernatant (liquid portion) contains your sample DNA, which will be used as your crude extract for PCR amplification in the next step. For long term storage, pipette the supernatant into a new, sterile tube and store at -20 ºC.

Note: You may also prepare the crude cell extract with the 1.5 mL tubes used to make the cell suspension and heat these tubes in a heat block set at 65 ºC for 6 minutes and 98 ºC for 2 minutes.

3. **Preparing the qPCR reaction mix and setting up the Real-Time PCR protocol:** Prepare a common qPCR mix for the total number of reactions that you plan to use. Table 1 shows the amount required for a single reaction. Make a common reaction mix without the DNA or crude cell extract. Determine the amount of each component required for the number of reactions that you plan to perform. Add an extra 10% of the volume for each component used. Add 20 µL of the reaction mix to each tube in the PCR strip. Set aside two tubes for the genotypes standards: 1) ACTN WT (RR genotype) and 2) ACTN Mutant (XX genotype). Prepare separate PCR reactions for each test or standard.

   The PCR strip tubes and strip caps can be cut and used for single reactions. Dilute the sample 1:1 as follows in a PCR tube: add 50 µL crude extract to 50 µL water (provided) and mix well by gently pipetting. Use either 5 µL of the 1:1 diluted crude extract or 5 µL of each of the standards for qPCR in a 25 µL reaction volume

   Use the protocol (Figure 2, below) for Real-Time PCR followed by a melt curve analysis. Ensure that Stage 2: Cycling is set for 40 cycles. The melt curve protocol can be added by selecting Add Stage under Edit Steps + Stages and selecting Melt Curve.

08

Components	1 Rx (µL)
Chai Green qPCR Master Mix	12.5
Primer Mix	5.0
Water	2.5
SUM	20.0

Table 1. Reaction setup

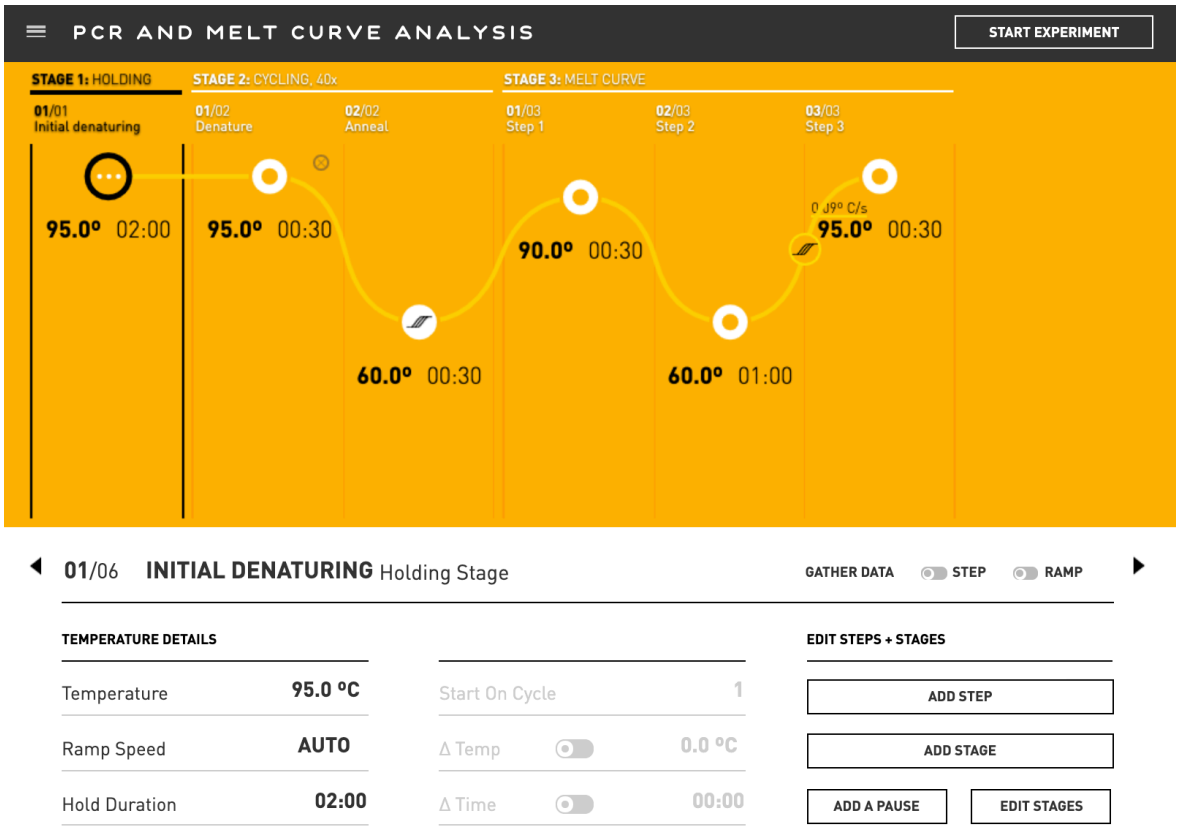

Figure 2. Real-Time PCR and melt curve analysis protocol

09 ANALYSIS

Once the run is complete, determine if there is any amplification by looking at the results page. Navigate to the Melt Curve chart. The SNP between the wild type and mutant genotypes of ACTN3 will alter the temperature that the target dsDNA will denature, resulting in two different melt curve peaks. The mutant genotype of ACTN3 will have a slightly lower melt temperature due to the weaker AT bond compared to the wild type genotype which has a stronger GC bond. Compare the shape and the peaks of the sample melt curves to the standards to determine the sample genotype. The shape of a heterozygous genotype sample will be distinct from that of wild type and mutant standards, as a heterozygous sample has both wild type and mutant genotypes and therefore has two melt temperatures (Figure 3).

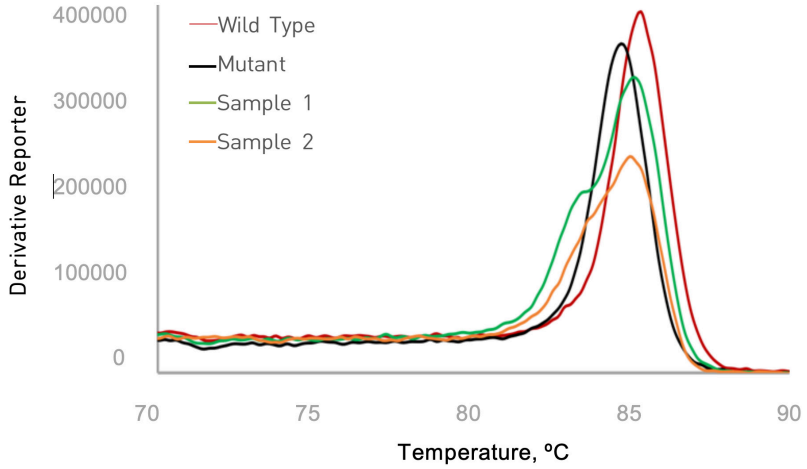

Figure 3. ACTN3 SNP genotyping using melt curve analysis. The melt curve of Wild Type standard of ACTN3 is shown in red and Mutant standard is shown in black. Samples 1 and 2 (green & orange) show curves like that of the heterozygous genotype.

10 REFERENCES

1. Michelle Mills, Nan Yang, Ron Weinberger, Douglas L. Vander Woude, Alan H. Beggs, Simon Easteal, and Kathryn North. Differential expression of the actin-binding proteins, α-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. Hum Mol Genet (2001) 10 (13): 1335-1346.
2. Sabrina Bernardez-Pereira, Paulo Caleb Junior Lima Santos, Jose Eduardo Krieger, Alfredo Jose Mansur, and Alexandre Costa Pereira. ACTN3 R577X polymorphism and long-term survival in patients with chronic heart failure. BMC Cardiovasc Disord. 2014 Jul 24;14:90.

11 SEQUENCES

Wild Type DNA:
GCACGATCAGTTCAAGGCAACACTGCCCGAGGCTGA**CCG**AGAGCGAGGTGCCATCATGGGCATCCAGGGTGAGATC
CAGAAG

Mutant DNA:
GCACGATCAGTTCAAGGCAACACTGCCCGAGGCTGA**CTG**AGAGCGAGGTGCCATCATGGGCATCCAGGGTGAGATC
CAGAAG

Forward Primer:
5'-TCAGTTCAAGGCAACACTGC-3'

Reverse Primer:
5'-CTTCTGGATCTCACCCTGGA-3'

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