CTNNB1 32-37 Mutation Analysis Kit

For quantitative detection of mutations in codons 32-37 in the *CTNNB1* gene by qPCR

50 tests

For research use only.

Developing Innovative, Noninvasive cfDNA tests for cancer screening and management

JBS SCIENCE

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Background

The human *CTNNB1* gene encodes Catenin beta-1 (or Beta-catenin), which has important functions in cell-cell adhesion and gene transcription. Beta-catenin is part of the Wnt signaling pathway, where it acts as a subunit of the cadherin protein complex. Mutations in *CTNNB1* that affect overexpression of Beta-catenin are largely observed in cancers such as hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and malignant endometrial cancer.

Nearly 10% of all cancers are found to contain *CTNNB1* mutations. Cancer causing mutations typically affect the N-terminal segment of Beta-catenin, which prevents degradation and ultimately results in Beta-catenin translocating to the nucleus to drive transcription of target genes. While there are several mutations reported, they are mostly localized between codons 32-45. This kit is capable of detecting mutations in codons 32-37, which make up nearly half of all *CTNNB1* mutations in cancer.

Kit Contents

P53 (249T) Mutation assay kit		**96 Reactions**
Tube Number	Reagent	Volume
①	STEP1 Oligo mix	150 µl
②	STEP2 Oligo mix	110 µl
③	pCTNNB1_32-37 plasmid 10^7 copies	10 µl
④	WT plasmid 10^7 copies	10 µl

Storage

The CTNNB1 32-37 Mutation analysis kit is shipped dried at room temperature. The entire contents may be stored at 4°C, and once resuspended in dH_2O should be properly stored at -20°C. It is recommended that stocks are kept at a 100µM concentration to maintain integrity.

Take care to protect the reagents from sunlight, and the STEP2 Oligo mix must be carefully protected from light.

Repeated freeze-thawing should be kept to a minimum. We recommend that if multiple reactions are to be run across multiple days, that the Oligos be aliquoted appropriately.

Product Use Limitations

The contents of this kit are intended for research purposes only. In no way or claim are the contents of this kit intended for diagnosis, monitoring, prevention, or treatment of a disease.

Due diligence is required for handling of the materials in this kit, and we recommend that NIH guidelines are adhered to in handling recombinant and isolated DNA.

This kit contains Oligo sets that can be used with any PCR or real-time PCR machine. We have validated this product with the Roche LightCycler® 480 System.

Materials and Equipment needed (not provided by this kit)

All standard safety procedures should be followed and protective equipment worn when working with chemicals, such as disposable gloves, protective eyewear, and a lab coat. Users should work in a lab fume hood in an area clear of contaminating PCR products.

The following reagents and materials do not come with this kit, and are necessary for using this kit:

Reagents

- STEP1 Master mix
  - dNTPs (recommended final concentration up to 2.5mM)
  - Polymerase with 10X buffer (recommended Qiagen HotStarTaq Plus DNA Polymerase Cat#203601)
- ExoMix
  - Exonuclease VII (recommended NEB Cat#MO379)
- STEP2 Master mix
  - LC480 probe master mix (recommended Roche Cat#04707494001; For use with Roche LightCycler480)
- CTNNB1 32-37 BNA^{NC}[NMe] (only from Bio-Synthesis Inc.)

Materials

- Standard pipets capable of dispensing 1-200 µl.
- Adjustable, sterile pipet tips (pipet tips with filters recommended)
- PCR Strip tubes with caps (0.2 ml)
- Microcentrifuge tubes for preparing master mix (1.5 ml)
- White polypropylene PCR plate (96-well)
- PCR Thermal Cycler
- Roche LightCycler® 480 System

Notes before getting started

- Ensure all equipment is calibrated
- Use a new pipet tip after every dispense
- Set up experiments as remote from areas of PCR product tube opening and gel loading as possible
- Thaw reagents on ice, and spin down once completely thawed
- Oligo mixes may be vortexed, but do not vortex enzymes or plasmid/genomic DNA (③④)
- Ensure samples are thoroughly mixed by vortexing or pipetting the mixture 10 times up and down
- Use a PCR cap tool for opening and closing the 0.2 ml tubes
- Make fresh dilutions of the standard for every use

Preparing the standards

This kit comes with two plasmids used as controls. The pCTNNB1_32-37 contains a mutation in codon 37, and the WT plasmid does not contain this mutation. Each standard comes with 10µl containing 10^7 copies/µl. A small aliquot should be taken for each run and diluted to the users preference. We recommend the following:

10,000 copies
1,000 copies
100 copies
10 copies

For each standard dilution it is recommended to also add the WT plasmid at a concentration similar to that of the samples that will be tested. For example, if 3ng of sample DNA is to be tested, we recommend 1000 copies of WT be added to each standard dilution (3ng ~ 1000 copies).

Procedure

1. **Prepare a reaction mix according to Table 1.**

Table 1. Step 1 reaction mix		
Reagent	**1X reaction**	**Master Mix (N reactions)**
① STEP1 Oligo mix	2.0µl	
❖CTNNB1 32-37 BNA^{NC}[NMe]	2.0µl	
*STEP1 Master mix	6.0µl	
dH_2O		
Sample DNA		

❖Must be purchased from Biosyn.com
*STEP1 Master mix is not included, but should include dNTPs, PCR buffer, and polymerase enzyme.
NOTE: Depending on amount of DNA added, dH_2O and STEP1 Master mix reagents can be scaled accordingly

Add reagents in order of top to bottom. For making a master mix, do not include sample DNA and for "N" reactions make 1 extra from what is needed to have sufficient volume.

2. **Prepare standards of the mutated CTNNB1 plasmid (pCTNNB1_32-37).**

Make 10, 100, 1,000, and 10,000 copies/µl stock dilutions of the plasmid.

3. **Remove the 8 well-strip tubes from the bag.**

4. **Dispense reaction mix into the 8 well-strip tubes.**

Add the appropriate amount (5-9µl) of STEP1 reaction mix (minus DNA sample and standards) into each well.

5. **Dispense standards into the reaction mix in the tubes as described in Table 2.**

Table 2	
(2µl) dH_2O	
(1µl) dH_2O	(1µl) ④ WT 1000 copies/µl
(1µl) ③ pCTNNB1_32-37 10 copies/µl	(1µl) ④ WT 1000 copies/µl
(1µl) ③ pCTNNB1_32-37 100 copies/µl	(1µl) ④ WT 1000 copies/µl
(1µl) ③ pCTNNB1_32-37 1,000copies/µl	(1µl) ④ WT 1000 copies/µl
(1µl) ③ pCTNNB1_32-37 10,000 copies/µl	(1µl) ④ WT 1000 copies/µl

6. **Dispense DNA samples into reaction mix in the tubes.**

Add 1-5µl of DNA sample into each well. (If using tissue we recommend 3ng of DNA; If using urine we recommend 1mL isolated DNA; If using serum we recommend 0.2mL isolated DNA). Final concentration of each well should be 10µl.

7. Mix samples and prepare for PCR.

Ensure samples have been mixed well by pipetting and centrifuge briefly.

8. Program the PCR cycle as described:

PCR: 95°C - 5min (95˚C - 30sec, 70˚C - 20 sec, 60˚C - 30sec) x 15cycles, 70˚C - 4min, 4˚C - hold

9. Run the PCR cycle.

10. Remove the tubes from the PCR machine and add 1 Unit of Exo-nuclease vii (NEB)

Once removed from the PCR machine, add 1 Unit of Exonuclease vii and mix well by pipetting up and down. NOTE – the enzyme can be diluted in buffer to add a larger volume for more accurate pipetting.

11. Program the PCR cycle as described:

PCR: 37°C - 60min, 95˚C – 10min, 60˚C – 1min (2% ramp), 4˚C – hold

12. Run the PCR cycle.

13. Remove the tubes from the PCR machine.

14. Prepare a reaction mix according to Table 3.

Table 3. Step 2 reaction mix		
Reagent	**1X reaction**	**N reactions**
dH_2O	2µl	
LC480 probe master mix	5µl	
②STEP2 Oligo mix	2µl	
STEP1 DNA exo-treated	1µl	

Add reagents in order of top to bottom. For making "N" reactions, make 1 extra from what is needed to have sufficient volume.

12. Remove a white 96-well qPCR plate from the sealed bag.

13. Dispense reaction mix into the qPCR plate.

Add 9µl of reaction mix into each well.

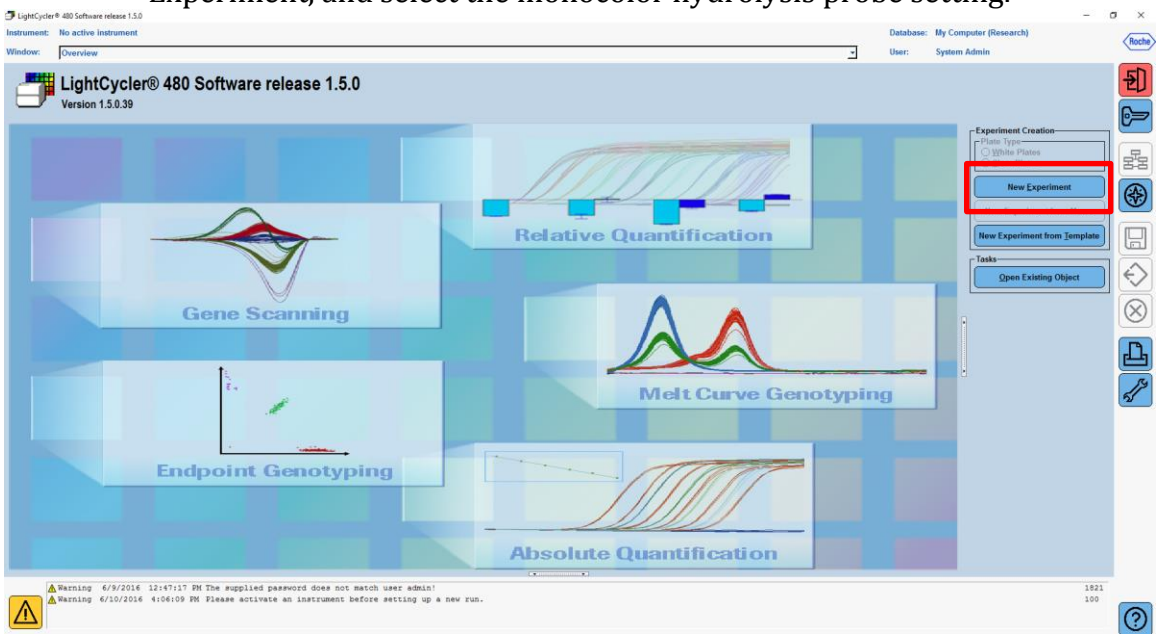
14. Dispense 1µl of amplified DNA (from step 13) into the qPCR plate.

15. Tightly seal the qPCR plate with adhesive film.
Gently push down around the edge of each well to create a tight seal. Centrifuge the plate on a benchtop centrifuge at 1000 rpm for 1 minute to remove any bubbles.

16. Program the PCR program into the Roche LC-480:

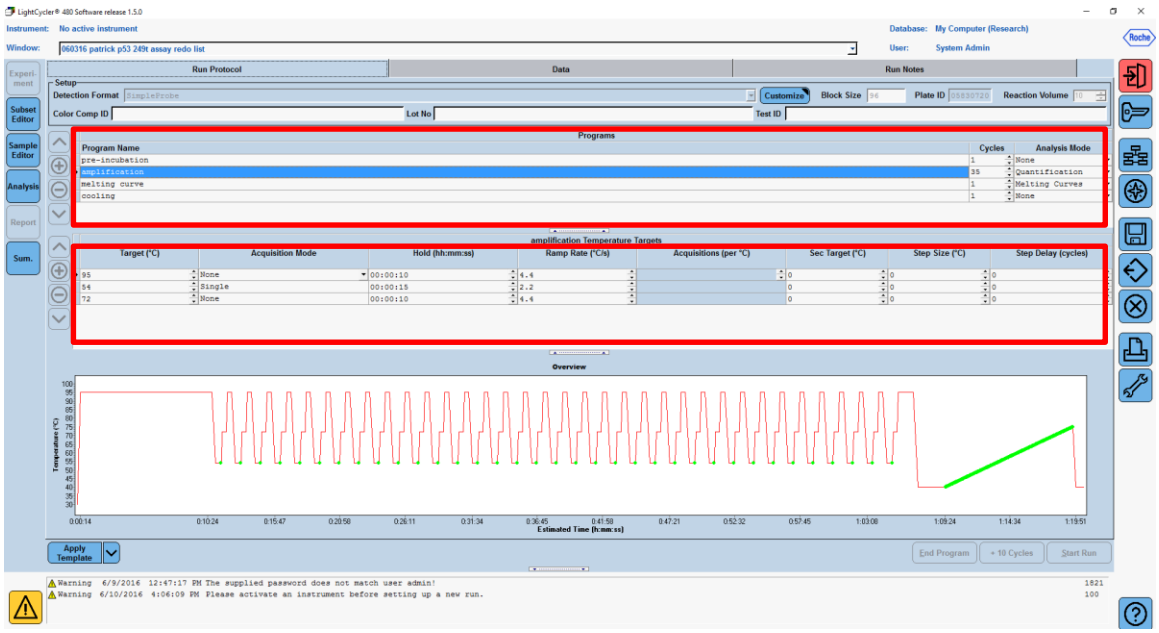
A. Open the program

On the main page of the Roche LightCycler® 480 Software, click "New Experiment, and select the monocolor hydrolysis probe setting.



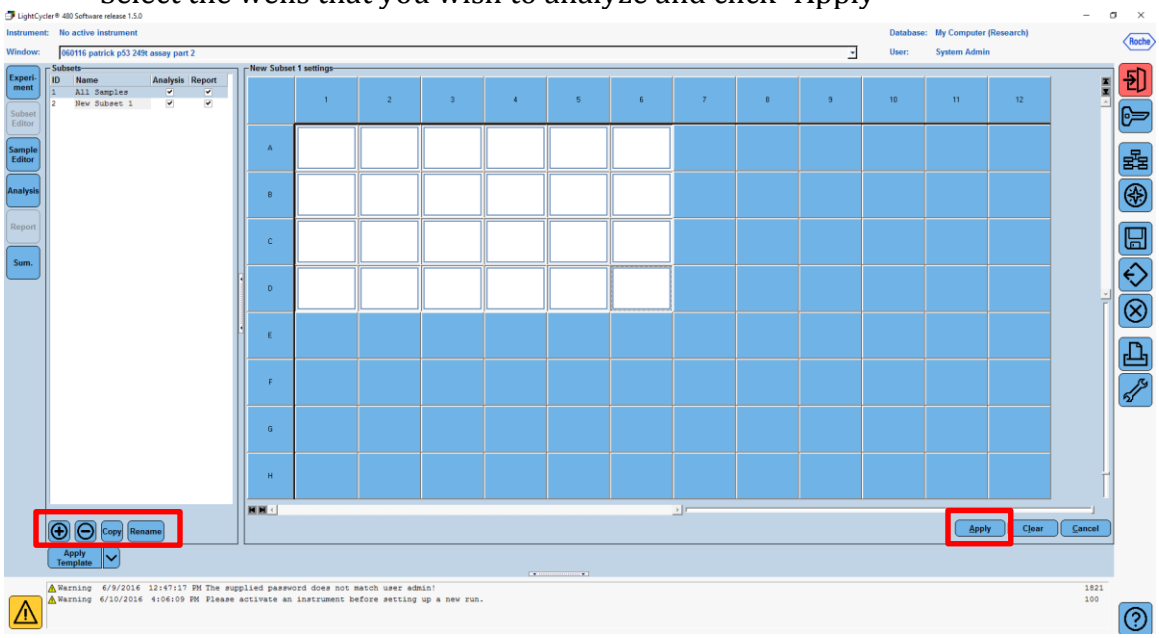
B. Fill out the following real-time PCR conditions on the Experiment tab:

Pre-incubation: 95°C - 10min
Amplification: 95°C - 10sec, 60°C - 10sec, 72°C - 10sec; 40cycles



C. Click the "+" button to create a new subset

Select the wells that you wish to analyze and click "Apply"



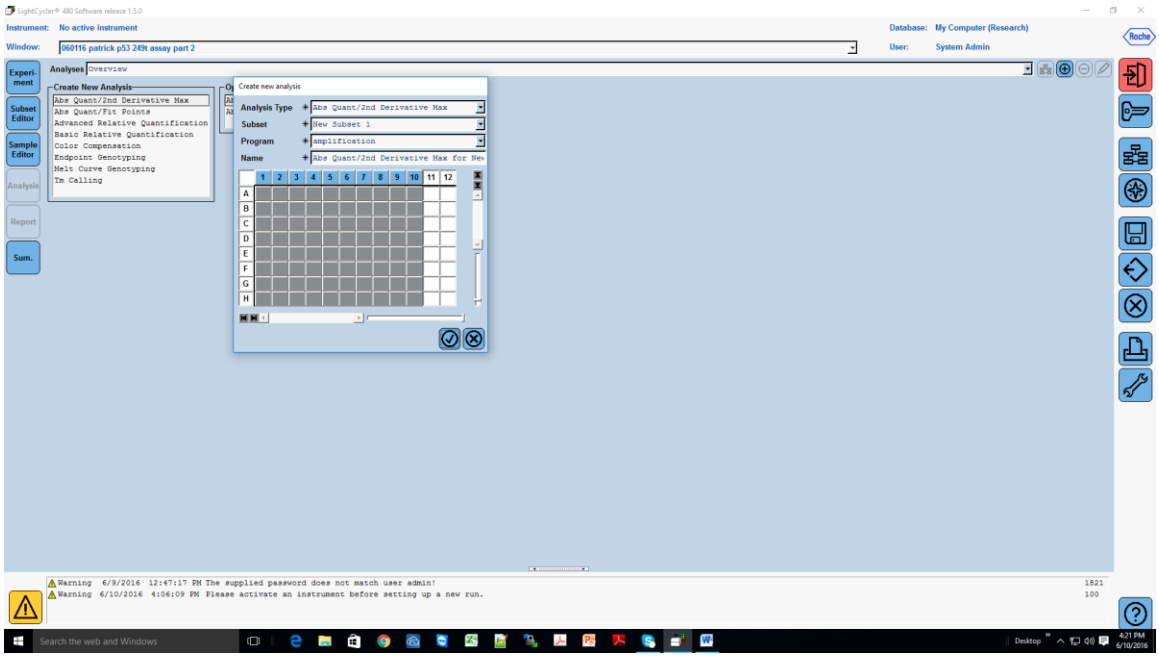
D. Fill out sample information in the sample editor tab.

For the standards, be sure to include the amount (i.e. copies).

E. Go back to the experiment tab and click Start Run.

18. Calculate the threshold cycle (C_T) for each well using the Roche LC-480 LightCycler software.

Click on the Analysis tab, and then select Abs Quant/2nd Derivative Max. Select your subset and then click the checkmark.



19. Export the C_T values to an Excel spreadsheet for data analysis.

Press the Calculate button to generate the Standard curve and provide the C_T values for each sample. Right-click on this table and export it as an Excel file for analysis and interpretation.

Assay sensitivity