Revision 11

Store at +4C

#29666

FastScan™ Cas9 (*S. pyogenes*) ELISA Kit

1 Kit (96 assays)

Species Cross Reactivity: All
UniProt ID: #Q99ZW2
Entrez-Gene Id: #901176

Orders: 877-616-CELL (2355) orders@cellsignal.com

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Product Includes	Product #	Quantity	Color
FastScan™ ELISA Microwell Strip Plate, 96 Well	53257	96 tests	
Cas9 (*S. pyogenes*) Rabbit Capture mAb	75578	1 ea	Green (Lyophilized)
Cas9 Mouse HRP-linked mAb	54725	1 ea	Red (Lyophilized)
FastScan™ ELISA Capture Antibody Diluent	16076	3 ml	Green
FastScan™ ELISA HRP Antibody Diluent	28120	3 ml	
TMB Substrate	7004	11 ml	
STOP Solution	7002	11 ml	
Sealing Tape	54503	1 ea	
ELISA Wash Buffer (20X)	9801	25 ml	
FastScan™ ELISA Cell Extraction Buffer (5X)	69905	10 ml	
FastScan™ ELISA Cell Extraction Enhancer Solution (50X)	25243	1 ml	
FastScan™ ELISA Kit #29666 Positive Control Type 2	93324	1 ea	

Kit contents scale proportionally with size, except sealing tape.
Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The FastScan™ Cas9 (*S. pyogenes*) ELISA Kit is a sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Cas9 (*S. pyogenes*). To perform the assay, sample is incubated with a capture antibody conjugated with a proprietary tag and a second detection antibody linked to HRP, forming a sandwich with Cas9 (*S. pyogenes*) in solution. This entire complex is immobilized to the plate via an anti-tag antibody. The wells are then washed to remove unbound material. TMB is then added. The magnitude of observed signal is proportional to the quantity of Cas9 (*S. pyogenes*).

*Antibodies in kit are custom formulations specific to kit.

IMPORTANT: This FastScan™ ELISA Kit requires 4 washes at Step 6 of the protocol.

Specificity/Sensitivity

The FastScan™ Cas9 (*S. pyogenes*) ELISA Kit detects endogenous levels of Cas9 (*S. pyogenes*), as shown in Figure 1. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

The CRISPR associated protein 9 (Cas9) is an RNA-guided DNA nuclease and part of the *Streptococcus pyogenes* CRISPR antiviral immunity system that provides adaptive immunity against extrachromosomal genetic material (1). The CRISPR antiviral mechanism of action involves three steps: (i), acquisition of foreign DNA by host bacterium; (ii), synthesis and maturation of CRISPR RNA (crRNA) followed by the formation of RNA-Cas nuclease protein complexes; and (iii), target interference through recognition of foreign DNA by the complex and its cleavage by Cas nuclease activity (2). The type II CRISPR/Cas antiviral immunity system provides a powerful tool for precise genome editing and has potential for specific gene regulation and therapeutic applications (3). The Cas9 protein and a guide RNA consisting of a fusion between a crRNA and a trans-activating crRNA (tracrRNA) must be introduced or expressed in a cell. A 20-nucleotide sequence at the 5' end of the guide RNA directs Cas9 to a specific DNA target site. As a result, Cas9 can be "programmed" to cut various DNA sites both *in vitro* and in cells and organisms. CRISPR/Cas9 genome editing tools have been used in many organisms, including mouse and human cells (4,5). Research studies demonstrate that CRISPR can be used to generate mutant alleles or reporter genes in rodents and primate embryonic stem cells (6-8).

Background References

1. Horvath, P. and Barrangou, R. (2010) *Science* 327, 167-70.

2. Wiedenheft, B. et al. (2012) *Nature* 482, 331-8.
3. Singh, P. et al. (2015) *Genetics* 199, 1-15.
4. Cong, L. et al. (2013) *Science* 339, 819-23.
5. Mali, P. et al. (2013) *Science* 339, 823-6.
6. Li, D. et al. (2013) *Nat Biotechnol* 31, 681-3.
7. Shen, B. et al. (2013) *Cell Res* 23, 720-3.
8. Niu, Y. et al. (2014) *Cell* 156, 836-43.

Trademarks and Patents

Limited Uses

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FastScan™ Cas9 (*S. pyogenes*) ELISA Kit

FastScan™ ELISA Protocol

A. Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent.
Prepare only as much reagent as needed on the day of the experiment.

1. **FastScan™ ELISA Microwell Strip Plate, 96 well (#53257)**: Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
2. **1X ELISA Wash Buffer**: Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
3. **1X Cell Extraction Buffer**: Prepare by diluting FastScan™ ELISA Cell Extraction Buffer (5X) #69905 and FastScan™ ELISA Cell Extraction Enhancer Solution (50X) #25243* to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1-2 weeks). To make 10 mL 1X Cell Extraction Buffer, combine 7.8 mL deionized water, 2 mL FastScan™ ELISA Cell Extraction Buffer (5X), and 200 µL FastScan™ ELISA Cell Extraction Enhancer Solution (50X). Alternatively, Enhancer Solution may be added to the Cell Extraction Buffer after extraction of cells or tissue. When using the 1X Cell Extraction Buffer as a sample diluent for the assay, it is recommended to equilibrate it to room temperature prior to use.

***IMPORTANT**: The provided FastScan™ ELISA Cell Extraction Enhancer Solution (50X) may precipitate when stored at 4°C. To dissolve, warm briefly at 37°C and mix gently. The FastScan™ ELISA Cell Extraction Enhancer Solution (50X) can be stored at room temperature to avoid precipitation.

NOTE: The 1X Cell Extraction Buffer contains phosphatase inhibitors. Protease inhibitors should be added to the 1X Cell Extraction Buffer immediately prior to lysing cells. Additional phosphatase inhibitors can also be added (e.g. Protease/Phosphatase Inhibitor Cocktail (100X) #5872, not supplied).

4. **FastScan™ ELISA Capture Antibody Diluent**: Green diluent for reconstitution of the Capture Antibody.
5. **FastScan™ ELISA HRP Antibody Diluent**: Diluent (amber bottle) for reconstitution of the HRP-linked Antibody. Protect from light.
6. **4X Capture Antibody**: Reconstitute lyophilized Capture Antibody (green colored cake) with 3 mL FastScan™ ELISA Capture Antibody Diluent (green diluent). Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted 4X Capture Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.
7. **4X HRP-linked Antibody**: Reconstitute lyophilized HRP-linked Antibody (red colored cake) with 3 mL FastScan™ ELISA HRP Antibody Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted 4X HRP-linked Antibody may be stored for up to 4 weeks at 4°C protected from light, although there may be some loss of signal compared to freshly reconstituted antibody.
8. **Antibody Cocktail**: Combine equal volumes of the reconstituted 4X Capture and 4X HRP-linked Antibodies immediately prior to assay and mix. To make 6 mL of the Antibody Cocktail (enough for 1x 96-well plate), combine 3 mL 4X Capture Antibody with 3 mL 4X HRP-linked Antibody.
9. **Positive Control**: Reconstitute 1 vial of lyophilized Positive Control (refer to product datasheet or vial label to determine which type of Positive Control is included with the kit):
    i. For Positive Control Type 1, add 250 µL deionized water.
    ii. For Positive Control Type 2, add 500 µL 1X Cell Extraction Buffer.

    Mix thoroughly and gently, hold at room temperature for 1 minute and then follow the steps outlined below in the "Test Procedure" section. Positive Controls are recommended to be used immediately after reconstituting, however remaining material may be stored at -80°C (there may be some loss of the positive control signal if freeze/thawed). Positive Controls are supplied as a control reagent, not as an absolute quantitation measure.
    NOTE: A select number of FastScan™ ELISA kits do not contain a positive control, please refer to Product Includes table on the datasheet for a list of included reagents. Should you need support on how to generate a positive control for those kits, contact CST technical support at support@cellsignal.com.
10. **TMB Substrate (#7004)**: Bring to room temperature before use.
11. **STOP Solution (#7002)**: Bring to room temperature before use.

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80-90% confluence.
2. Remove media and rinse cells once with ice-cold 1X PBS.

3. Remove PBS and add 0.5 mL ice-cold 1X Cell Extraction Buffer (recommended to supplement with protease inhibitors and additional phosphatase inhibitors as needed) to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 5 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at −80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5-1.0 x 10^6 viable cells/ml.
2. Wash once with ice-cold 1X PBS.
3. Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Extraction Buffer (recommended to supplement with protease inhibitors and additional phosphatase inhibitors as needed).
4. Sonicate lysates on ice.
5. Microcentrifuge for 5 min (x14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at −80°C in single-use aliquots.

C. Test Procedure

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Samples should be undiluted or diluted with 1X Cell Extraction Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the antibody cocktail. Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical results across a range of lysate concentration points.
3. Add 50 µL of each sample or Positive Control to the appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate with the supplied sealing tape and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
6. Gently remove the tape and wash wells:
    a. Discard plate contents into a receptacle.
    b. Wash 3 times* with 1X ELISA Wash Buffer, 200 µL each time for every well. After each wash, aspirate or decant from wells. Invert the plate and blot it against clean paper towels to remove the residual solution in each well, but do not allow wells to completely dry at any time.
    c. Clean the underside of all wells with a lint-free tissue.

***NOTE**: Certain FastScan™ ELISA Kits may require additional washes at this step. Any requirements for additional washes will be specifically noted in the product “Description” of the kit’s datasheet.

7. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
8. Add 100 µL of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

9. Read results:
    a. **Visual Determination**: Read within 30 min after adding STOP Solution.
    b. **Spectrophotometric Determination**: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

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