G3-YSD Control

Negative control for G3-YSD

Catalog code: tlrl-ydnac

http://www.invivogen.com/g3-ysdc

For research use only

Version 18E17-NJ

PRODUCT INFORMATION

Contents

• 200 µg G3-YSD Control (C3-ended Y-form Short DNA)
• 1.5 ml sterile endotoxin-free water

Sequence

5' **CCC** TATATATATGCATATATATA **CCC** 3' (26 mer)

Note: G3-YSD Control contains a palindromic sequence for which self-hybridization results in double-stranded DNA. The flanking cytidine trimers in 5' and 3' remain unpaired.

Molecular weight

7848.3 g/mol

Storage and stability

- G3-YSD Control is provided lyophilized and shipped at room temperature. Store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 12 months at -20 °C. Avoid repeated freeze-thaw cycles.

Quality control

- The inability to induce type I interferon has been verified using cellular assays.
- The absence of bacterial contamination, such as lipoproteins and endotoxins, has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

G3-YSD Control, is a 26-mer DNA sequence derived from the HIV-1 RNA genome which is used as a negative control for G3-YSD, a potent agonist of the critical cytosolic DNA sensor cGAS (cyclic GMP-AMP synthase, cGAMP synthase)[1]. These two palindromic sequences differ in the flanking nucleoside trimers in 5' and 3'. While G3-YSD is flanked with guanosine trimers (G3) confering its agonist activity, G3-YSD Control is flanked with cytidine trimers (C3) abrogating cGAS activation[1]. cGAS detects double-stranded DNA (dsDNA) over 40 bp in length (ISD recognition), or stem-loop structures of single-stranded DNA (ssDNA) flanked by unpaired nucleotides (YSD recognition)[1-3]. Interaction of cytosolic DNA with cGAS promotes the synthesis of 2'3'-cGAMP, a second messenger that activates STING (stimulator of interferon genes), and the downstream production of type I interferons (IFNs) and other cytokines[2].

Unlike G3-YSD, intracellular delivery of the G3-YSD Control cytidine-flanked sequence does not induce type I IFN production.

1. Herzner AM. *et al.*, 2015. Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. Nat Immunol. 16(10):1025-33. **2. Li T. & Chen ZJ., 2018.** The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. J Exp Med. **3. Luecke S. *et al.*, 2017.** cGAS is activated by DNA in a length-dependent manner. EMBO Rep. 18(10):1707-1715.

METHODS

Preparation of G3-YSD Control stock solution (1 mg/ml)

- Add 200 µl of sterile endotoxin-free water (provided) to 200 µg of G3-YSD Control.
- Vortex gently until completely dissolved.

Working concentration: 100 ng - 1 µg/ml

Below is a protocol for cGAS stimulation upon intracellular delivery of G3-YSD. Note that G3-YSD Control does not activate cGAS.

cGAS stimulation using G3-YSD and LyoVec™ transfection reagent in THP1-Dual™ reporter cells

THP1-Dual™ reporter cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF (interferon regulatory factor) pathway, by assessing the activity of secreted Lucia luciferase. These cells derive from the human THP-1 monocytic cell line which is often used to study DNA sensing pathways. Indeed, THP-1 cells express all the cytosolic DNA sensors identified so far (with the exception of DAI).

To achieve cGAS stimulation, G3-YSD must be delivered to the cytoplasm, for example by using a transfection agent, such as LyoVec™. Use G3-YSD Control with the same intracellular delivery system and concentration as for G3-YSD.

1. Rehydrate LyoVec™, G3-YSD and G3-YSD Control at the recommended concentrations. Bring reagents to room temperature and mix gently to homogenize before use.
2. In a sterile 1.5 ml microfuge tube at room temperature, mix 1 µl (1 µg) of G3-YSD or G3-YSD Control stock solution (1 mg/ml) with 100 µl of LyoVec™. Mix gently.
3. Incubate at room temperature for 15-30 minutes to allow the formation of the complex.
4. Add 20 µl of G3-YSD/LyoVec™ or G3-YSD Control/LyoVec™ complex (100 ng - 1 µg/ml final concentration) to each well of a 96-well plate.
5. Distribute 180 µl of a THP1-Dual™ cell suspension (100,000 cells per well).
6. Incubate for 24-48 hours at 37 °C.
7. Determine cGAS stimulation by assessing Lucia luciferase reporter gene expression using QUANTI-Luc™ .

RELATED PRODUCTS

Product	Catalog Code
G3-YSD	tlrl-ydna
2'3'-cGAMP	tlrl-nacga23
THP1-Dual™ Cells	thpd-nfis
THP1-Dual™ KO-cGAS Cells	thpd-kocgas
THP1-Dual™ KO-STING Cells	thpd-kostg
LyoVec™	lyec-12
VACV-70	tlrl-vav70n

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