



#### Sep 08, 2020

# FLASH amp

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<sup>1</sup>Quantification by Design, Stanford University

1 Works for me dx.doi.org/10.17504/protocols.io.bk2hkyb6

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#### ABSTRACT

FLASH amp is a method that allows for room temperature detection and amplification of a viral RNA sequence of interest submitted as part of the Xprize competition. The main components of the reaction are not included here and are given generic names such as enzyme X as they are currently not protected under IP. The steps outlined with tubes labeled with the generic names would allow any lab to reproduce the method.

Version 1 of this method outlines the protocol for a fluorescence-based readout that can be used with a qPCR machine or TECAN style plate reader.

Version 2 (to be released shortly) will be a colorimetric readout that can be assessed by eye.

The outlined protocol assumes the user performing this in a laboratory setting will setup reactions in a clean room and analyze results in a seperate post-amplification room. In addition, since our amplification is rapid and at room temperature, care but be taken to add ligand to the master mix quickly and the reactions be sealed.

DOI

dx.doi.org/10.17504/protocols.io.bk2hkyb6

PROTOCOL CITATION

eesha.sharma.phd 2020. FLASH amp. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bk2hkyb6

**KEYWORDS** 

**Xprize** 

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41769

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Pyrophosphatase, Inorganic (E.coli) - 50 units	M0361L	New England Biolabs
Corning® Low Volume 384-well Black Flat Bottom Polystyrene	3821BC	

FOLIPMENT



09/08/2020

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NAME CATALOG # VENDOR
SPARK SPARK

DISCLAIMER:

This method is currently used for research and development, not for diagnostic purposes.

## Sample collection

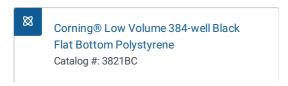
Sample should be collected 1 hour after any food is consumed.
 minutes before collection rinse mouth well with water.
 Collect passive drool or pooled salive below tongue into collection tube.

### Set-up

- 2 Resuspend freeze dried master mix 1 with **□7.04 μl** of water
- 3 Resuspend freeze dried master mix 2 in 📮 1 🏚 water and add to above
- 4 Add **□0.3** µl
  - Pyrophosphatase, Inorganic (E.coli) 50 units
    by New England Biolabs
    Catalog #: M0361L
- 5 Add **□0.5** μl

Undisclosed enzyme Z

- 6 Add **□0.5 μl** Undisclosed Enzyme Y
- 7 Add □0.13 μI Undisclosed Enzyme X
- 8 Put mix in



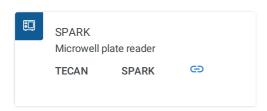
Add  $\mathbf{0.5} \, \mu \mathbf{l}$  of sample and seal plate.



A large master mix of the above components can be mixed and aliquoted into wells.

## Data collection

9 Place plate in TECAN plate reader.



Set gain to 120, excitation 480 and emission 550.

A qPCR machine can also be used.

Collect data at T= 30 min.

Data can also be collected every 1s for 30 min to monitor kinetics of reaction in the particular plate reader being used if not TECAN SPARK reader.

Fluorescence values of >4000 are positive and <4000 are negative.

