

# SoliSD™ Bsm DNA polymerase Kit

Catalogue Number	Size
32-21-0000S	100 reactions
32-21-00250	250 reactions
32-21-01000	1000 reactions



## Shipping:

At room temperature

## **Batch Number and Expiry Date:**

See vial

Store at –20°C upon receipt

## Storage and Stability\*:

- Routine storage at -20°C (-28°C to -18°C) until Expiry Date
- Stable at 4°C (2°C to 8°C) for up to 6 months
- Stable at room temperature (15–25°C) for at least 1 month
- Freeze-thaw stability: 10 cycles

## Reaction setup:

At room temperature

Manufactured by Solis BioDyne, in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

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### **Product description:**

- SoliSD™ Bsm DNA polymerase is based on the Large Fragment of the DNA polymerase from the genus Bacillus smithii with functional similarity to other Bst and Bsm DNA polymerases. It has enhanced stability due to the incorporated Stability TAG (covered by the patent EP2501716)\*\*.
- The enzyme has a strong strand displacement DNA polymerase activity and lacks 5'→3' and 3'→5' exonuclease activities. It is designed for applications requiring synthesis through double-stranded DNA regions, such as loop-mediated isothermal amplification (LAMP).
- Unique SoliSD™ Supplement system:
  - o Resolves common NTC signal issue
  - o Minimizes variability between replicates
  - Allows reaction set-up at room temperature
- SoliSD™ Bsm DNA polymerase Kit is provided in a flexible 5-vial format. The development of an isothermal amplification assay can be challenging, and optimization depending on the target, primer set, and detection method is often required.

#### **Features**

- 1. Enzyme is active at a wide temperature range between 51-62°C with optimum at 60°C
- **2.** No  $5' \rightarrow 3'$  and  $3' \rightarrow 5'$  exonuclease activity
- 3. Strong strand displacement DNA polymerase activity
- 4. Short 4-20 minute reaction time to result
- 5. No NTC signal
- 6. Increased thermostability for at least 1 month at 37°C'
- 7. Market-level inhibitor tolerance

### Reagents supplied:

	Catalogue Number		
Component	32-21- 0000S	32-21- 00250	32-21- 01000
SoliSD™ Bsm DNA polymerase (8 U/µl)	100 µl	250 µl	1 ml
10x Isothermal Reaction Buffer (pH 8.8); 200 mM Tris-HCl, 150 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ,1% Tween-20, 250 mM KCl	1 ml	1 ml	3 ml
100 mM MgSO <sub>4</sub>	500 µl	500 µl	2 ml
25x SoliSD™ Supplement	250 µl	250 µl	1 ml
10x GC-rich Enhancer	500 μl	1 ml	3 ml

## Step-by-step guidelines:

- **1.**Thaw the reagents at room temperature. Mix by vortexing or pipetting up and down, then centrifuge briefly.
- 2. Prepare a reaction master mix. Add components except for template DNA in the suggested order. Mix by vortexing or pipetting up and down, then centrifuge briefly.

Reaction component	Volume	Final conc.
Nuclease-free water <sup>1</sup>	Up to 25 µl	
10x Isothermal Reaction Buffer	2.5 μl	1x
100 mM MgSO <sub>4</sub>	1.6 µl	6.4 mM <sup>a</sup>
10x GC-rich Enhancer <sup>2</sup>	optional	optional
dNTPs <sup>1</sup>	variable	1-2 mM
25x SoliSD™ Supplement	1 μl	1x
SoliSD™ Bsm DNA polymerase	1 μl	0.32 U/µl
Fluorescent Dye <sup>1</sup>	variable	0.3-0.5 μΜ
FIP/BIP primers <sup>1</sup>	variable	1.6 µM
F3/B3 primers <sup>1</sup>	variable	0.2 μΜ
LoopF/B primers <sup>1</sup>	variable	0.4 μΜ
Template DNA <sup>1</sup>	variable	10 <sup>1</sup> -10 <sup>10</sup> cp/rxn
Total	25 μl	

 $<sup>^{1}</sup>$  Not included in the kit;  $^{2}$  Addition of GC-rich Enhancer is optional, consider adding 1x, 2x, or 5x final concentration to the reaction mix in case of a GC-rich template.

<sup>&</sup>lt;sup>a</sup> Optimal concentration of MgSO<sub>4</sub> in LAMP is between 6-7 mM.

- 3. Dispense the reaction mixture and add DNA template.
- **4.**Perform the reaction at 60°C for 10-45 min. SoliSD™ Bsm DNA polymerase is active at 51-62°C. The optimal temperature of the assay may vary depending on the primers and final buffer conditions.
- **5.** If need be, inactivate the enzyme by heating it at 80°C for 10 min.

### Primer design and validation

LAMP assay requires a set of 4-6 primers. We recommend screening several sets of primers to achieve the best sensitivity and eliminate the possibility of the signal in an NTC (no-template control) reaction. To facilitate primer design use dedicated primer design software.

#### Contamination control

To prevent contamination, we recommend the separation of working areas into pre-amplification (reaction mixture preparation), amplification (dispensing of reaction mixture and addition of template), and post-amplification. Note! Due to the nature of enzyme and primers used in LAMP reactions, opening reaction vessels following amplification is not recommended.

#### Controls

We recommend including several controls:

- Negative controls: no template and non-target template controls.
- Positive control: a previously validated template and primer set.

## **Troubleshooting guide**

### No template or non-target template signal

- Clean all surfaces with DNase-containing solution.
- Substitute reagents and consumables.
- Add primers last while preparing the master mix. Do not incubate mix with primers for a prolonged time.
- Reduce reaction time.
- Inactivate enzyme if the result read out is performed postreaction (e.g. LF, pH-sensitive methods).

### Time-to-result is not optimal

- Use nucleotides diluted in buffer without TE.
- Revise sample preparation method. Consider heat treatment and the addition of GC-rich Enhancer.
- $\bullet$  If EDTA is added to the reaction mixture, neutralize its inhibition effect by the addition of MgSO<sub>4</sub> in a 1:1 ratio.
- Optimal MgSO<sub>4</sub> concentration is at 6.4 mM. Depending on your assay design, titrate concentration at range 6-7 mM.
- Multiplex reaction assay may require more optimization and scaling up of different components. Start with dNTP and primers concentration optimization.

### Too low or too high fluorescence level

- Use recommended fluorescent dye and optimize concentration if required:
  - $\circ~$  SYTO9: 0.2-0.5  $\mu M$  (optimal 0.35  $\mu M)$
  - o Chai: 0.2-0.5 μM (optimal 0.3 μM)
  - O EvaGreen:  $1x = 0.7 \mu M$

#### Source:

Purified from an E. coli strain that carries an overproducing plasmid containing a SoliSD<sup>TM</sup> Bsm DNA polymerase gene.

#### **Unit definition:**

The unit is determined in DNA strand displacement assay, where 1 unit of the enzyme catalyzes displacement of 40 nmol of oligonucleotide probe from the complementary DNA strand in 30 min at 60 °C.

### Safety precautions:

Please refer to Safety Data Sheet for more information.

# Technical support:

Contact your sales representative for any questions or send an email to <a href="mailto:support@solisbiodyne.com">support@solisbiodyne.com</a>

For research use only. Not for use in diagnostic procedures.

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\*Product stability is assessed using routine QC assays and QC criteria set forth in the product specification and are intended to provide guidelines for shipping and storage conditions only. Customer or its designee shall be responsible for conducting all necessary stability testing applicable to their assay and/or QC criteria, and to comply with any applicable regulatory requirements or guidelines. Such stability testing shall include testing to validate the lead times for shipment, the shelf life of, and the product specifications applicable to shipment, storage and handling of the assay assembled and packed by the customer.

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\*\*Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

**Trademark information:** SoliSD™ is a trademark of Solis BioDyne OÜ.

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