# Bsal, GMP-Grade

# Catalog #GMP-BSA-EE101

**Storage Condition**  $-20^{\circ}C \pm 5^{\circ}C$  for 24 months. Avoid repeated freeze/thaw cycles.

Form Liquid

Source E. coli

Storage Buffer 10mM Tris-HCI, 200mM NaCl, 50% Glycerol, 0.1mM EDTA, 1mM DTT, pH 7.4

Concentration 20U/µL

**Unit Definition** One unit is defined as the amount of enzyme required to digest 1  $\mu$ g of pXba DNA in 1 hour at 37°C in a total reaction volume of 50  $\mu$ L.

#### Special formulation enables BSA-free reaction.

## **Product Contents**

- Bsal (20U/µL)
- 10X Cut Reaction Buffer (200 mM Tris-acetate, 500 mM Potassium Acetate, 100 mM Magnesium Acetate, 1 mg/ml Recombinant Albumin, pH 7.9)

#### **Product Description**

Bsal is designed to be used upstream of *in vitro* mRNA synthesis for mRNA therapeutics and vaccine manufacturing. As one of the type IIS restriction enzymes, it recognizes asymmetric DNA sequences and cleaves outside of their recognition sequence. The cleavage site of Bsal is GGTCTC(N1/N5), where the GGTCTC acts as the recognition site, and the N1/N5 represents the cleavage site with a 5' overhang. KACTUS provides a BSA-free reaction buffer to ensure the safety of mRNA vaccine production.

## Applications

- Molecular cloning
- Genotyping
- SNP
- Plasmid Linearization

#### **Quality Control Statement**

This product has been filed with the FDA Drug Master Files and is assigned DMF #037503. KACTUS manufactures this product according to GMP guidelines and performs stringent quality control testing before release. The production is antibiotic- and animal-free.

Quality Control Release Criteria								
Assay	Criteria							
Activity (Digestion activity detection)	≥ 20kU/mL							
Purity (SEC-HPLC)	≥ 95%							
Residual Endonuclease	Negative							
Residual Exonuclease	Negative							

Endotoxin	≤ 10EU/mL			
Residual DNase	Negative			
Residual RNase	Negative			
Residual Protease	Negative			
Residual Host Cell Protein	≤ 20ng/mg			
Residual Host Cell DNA	≤ 100pg/mL			
Residual Heavy Metal	≤ 10ppm			
Bioburden	≤ 1CFU/10mL			

## Protocol for Restriction Digest

1. Prepare the following reaction mixture:

Reagent	Quantity			
10X Cut Reaction Buffer	2µL			
DNA	2µL (up to 1µg)			
Bsal (20 U/μL)	1µL			
RNase-free Water	Up to 20µL			

- 2. Mix gently and spin down for a few seconds.
- 3. Incubate at 37°C for 15 minutes.
- 4. (Optional) Inactivate Bsal by incubating at 80°C for 20 minutes.

#### Notes

• Bsal is sensitive to Dam, Dcm, or CpG methylation.

## **Performance Validation**

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Results of a 30-minute digest reaction at 37°C with 1µg plasmid and 1µL Bsal (2-fold serial dilution in reaction buffer from lane 1 to lane 11, with lane 1 from 1 µL 20U of Bsal, lane 2 from 1 µL 10 U of Bsal, and so on) in a total reaction volume of 20µL. N is non-digested plasmid.