Sph I

GCATG^C

AccuCut™ *Restriction Endonuclease*

• **Cat. No** **E-2071** **200 Units**
E-2072 **1000 Units**

• **Lot No. : 01 C27451H8A3**

• **Supplied with Enzyme**

10X AccuCut™ Orange Buffer : 1 mL

100 mM	pH 7.6 Tris-HCl
100 mM	$MgCl_2$
500 mM	NaCl
10 mM	DTT

1X Dilution Buffer : 1 mL

10 mM	pH 7.6 Tris-HCl
50 mM	KCl
0.1 mM	EDTA
1 mM	DTT
200 μg/mL	Acetylated BSA
50%	Glycerol

• **Store at -20℃.**

• **Unit definition** : One unit of restriction endonuclease activity is defined as the amount of enzyme required to completely digest 1μg of substrate DNA in a total reaction volume of 50 μL in one hour using the AccuCut™ buffer provided. Incubations are performed in 1.5 mL tubes at the appropriate incubation temperature as indicated in the Product Profile.

• **Isoschizomer** : *Bbu* I ,*Pae* I.

• **Neoschizomer** :Unfound

• **Reactivity on methylated substrate DNA:** Blocked by GC ^{m6}ATGC, G^{hm5}CATG^{hm5}C. Not blocked by GCATG^{m5}C.

• **Ref)** 1. Bhattacharya, S.K., Dubey, A.K., (1994) Biotechnol. Appl. Biochem., vol. 20, pp. 141-146.
2. Fuchs, L.Y., Covarrubias, L., Escalante, L., Sanchez, S., Bolivar, F., (1980) Gene, vol. 10, pp. 39-46.

• **Source :** ***Streptomyces phaeochromogenes.***

• **Concentration : 4 Units/ μL**

• **Reaction Condition**

- 10X AccuCut™ **O**range **Buffer** Buffer
- Incubate at 37℃.

•**Storage Buffer**

20 mM	pH 7.5, Tris-HCl
50 mM	KCl
1 mM	EDTA
10 mM	2-mercaptoethanol
50%	Glycerol

• **Heat inactivation :** 65℃ for 20 minutes.

Quality Control

• **Overdigestion Assay :**
No nonspecific activity was detected after incubation of 1 μg of λ DNA with 50 units of *Sph* I for 15 hours.
* Conditions of low ionic strength, high enzyme concentration, glycerol concentration >5%, or pH >8.0 may result in star activity.

• **Nuclease Contamination Assay :**
No altered pattern was detected after incubation of 1 μg of substrate DNA with *Sph* I in 50 μL reaction volume with the supplied AccuCut™ buffer overnight.

• **Ligation and Recutting Assay :**
This assay is used to test for exonuclease activity that would degrade the termini of restriction fragments, resulting in inhibition of ligation and of subsequent digestion of ligated fragments. After 40-fold overdigestion with *Sph* I, 95% of the DNA fragments can be ligated and recut with *Sph* I.