


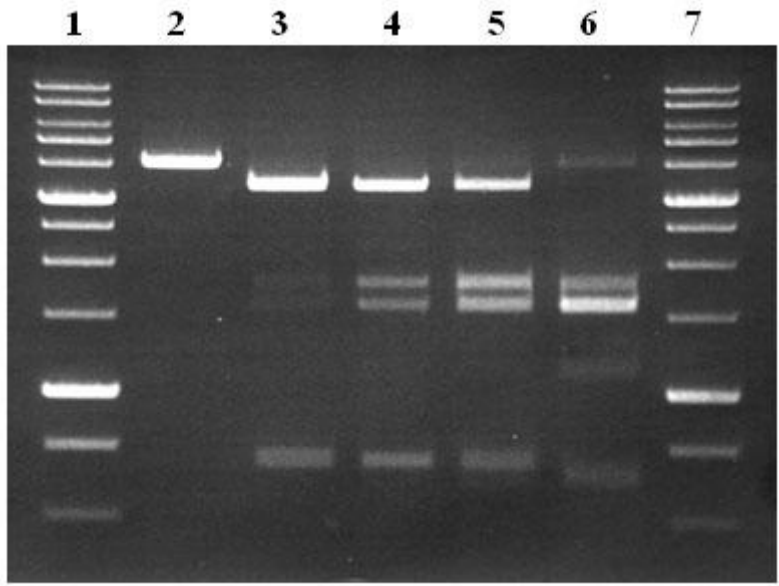


DATA SHEET

Product info: Gla I


Name	Gla I 	
Cat. #	E493	E494
Package, u.a.	100	500
Concentration, u.a./ml	8000	8000

Recognition site	<p>Pu(5mC)↑GPy PyG↓(5mC)Pu</p>
Source	Glacial ice bacterium GL29
Substrate specificity	<p>The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA and DNA with N4-methylcytosines.</p> <p>The enzyme activity depends on number and position of methylated nucleotides in the recognition sequence:</p> <p>Optimal substrate (100% activity) 5`-G(5mC)G(mC)-3`/3`-(5mC)G(5mC)G-5`</p> <p>Good substrates (> 25% activity) 5`-R(5mC)G(5mC)-3`/3`-YG(5mC)G-5` 5`-A(5mC)GT-3`/3`-TG(5mC)A-5`</p> <p>Medium substrates (> 6% activity) 5`-G(5mC)R(5mC)-3`/3`-(5mC)GYG-5` 5`-G(5mC)GT-3`/3`-CG(5mC)A-5`</p>


	<p>Bad substrates (6% activity) $5\text{'-G(5mC)GC-3'}/3\text{'-CG(5mC)G-5'}$</p>
<p>Assayed on</p>	<p>DNA pHspAI2/Gsal is a linearized plasmid pHspAI2, which carries a gene of DNA-methyltransferase M.HspAI (recognition sequence 5'-GCGC-3') and includes a unique GlaI recognition site $5\text{'-G(5mC)G(5mC)-3'}/3\text{'-(5mC)G(5mC)G-5'}$.</p>
<p>Unit definition</p>	<p>One unit is defined as the amount of enzyme required to hydrolyze completely a unique $5\text{'-G(5mC)G(5mC)-3'}/3\text{'-(5mC)G(5mC)G-5'}$ site in 1 μg of pHspAI2 plasmid DNA, which is linearized with Gsal, in 1 hour at 30°C in a total reaction volume of 50 μl. As a result of this site hydrolysis two DNA fragments are produced (see run 3 in the figure). GlaI digestion of recognition sequences with three and two 5-methylcytosines results in additional bands appearance (runs 4-6 in the figure).</p>
<p>GlaI activity assay on DNA pHspAI2/Gsal</p> <p>Lanes: 2 – Control DNA pHspAI2/Gsal, 3 – 0.5 μl Gla I (1/10) 4 – 1 μl Gla I (1/10) 5 – 2 μl Gla I (1/10) 6 – 1 μl of undiluted GlaI 1 and 7 – 1 Kb SE DNA-markers.</p> <p>Products were separated in</p>	




DATA SHEET


1% agarose gel in Buffer TAE.	
Reaction buffer	SE-buffer Glal , (10 mM Tris-HCl (pH 8,5 at 25°C); 5 mM MgCl ₂ ; 10 mM NaCl; 1 mM 2-mercaptoethanol.)
Optimal temperature	30°C
Storage conditions	10 mM Tris-HCl (pH 7.5); 200 mM NaCl; 0.1 mM EDTA; 7 mM 2-mercaptoethanol; 0,05% Triton X-100, 0.1 mg/ml BSA, 50% glycerol; Store at -20°C.
Non-specific hydrolysis	No detectable degradation of 1µg of Lambda DNA was observed after incubation with 8 units of enzyme for 16 hours at 30°C in a total reaction volume of 50 µl.
Reagents Supplied with Enzyme	10 X SE-buffer Glal, pHspAl2/Gsal DNA
Methylation sensitivity	The enzyme cleaves only C5-methylated DNA and does not cut unmodified DNA and DNA with N4-methylcytosines .
Inactivation 20 minutes under	65°C
References:	<ol style="list-style-type: none">1. <i>Chernukhin V.A., Nayakshina T.N., Tomilova J.E., Mezentseva N.V., Dedkov V.S., Degtyarev S.Kh. Bacterial strain Glacial ice bacterium I - producer of Glal restriction endonuclease.</i> // Russian Federation patent RU 2287012 C1 (2006).2.  <i>Valery A. Chernukhin, Tatyana N. Najakshina, Murat A. Abdurashitov, Julia E. Tomilova, Nina V. Mezentzeva, Vladimir S. Dedkov, Natalya A. Mikhnenkova, Danila A. Gonchar, Sergei Kh. Degtyarev A novel</i>

restriction endonuclease Glal recognizes methylated sequence 5'-G(m5C)^{AGC}-3' // *Biotechnologia (russ.)*. 2006. N 4. P. 31-35


3.  **Tomilova J.E., Chernukhin V.A., Degtyarev S.Kh. Dependence of site-specific endonuclease Glal activity on quantity and location of methylcytosines in the recognition sequence 5'-GCGC-3'**. // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.2, No 1, pp 30-39 (2006) (Russian)

4.  **G. V. Tarasova, T. N. Nayakshina, S. Kh. Degtyarev Substrate specificity of new methyl-directed DNA endonuclease Glal** // [BMC Molecular Biology 2008, 9:7](#)

Application:


 **Chernukhin V.A, Abdurashitov M.A., Tomilov V.N., Gonchar D.A., Degtyarev S.Kh. Comparative analysis of mouse chromosomal DNA digestion with restriction endonucleases in vitro and in silico** // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.3, No 4, pp 19-27, 2007

Abdurashitov M.A., Chernukhin V.A, Gonchar D.A., Degtyarev S.Kh. Glal digestion of mouse γ -satellite DNA: study of primary structure and ACGT sites methylation // [BMC Genomics 2009, 10:322.](#)

 **D. A. Gonchar, A. G. Akishev, S. Kh. Degtyarev BslI- and Glal-PCR assays – a new method of DNA methylation study** // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.6, No 1, pp 5-12, 2010

Wood, R. J., McKelvie, J. C., Maynard-Smith, M. D., and Roach, P. L. A real-time assay for CpG-specific cytosine-C5 methyltransferase activity.// (2010) *Nucleic Acids Res* 38, e107

Kravets AP, Mousseau TA, Litvinchuk AV, Ostermiller Sh, Vengen GS, Grodzinski DM. Changes in wheat DNA methylation pattern after chronic seed gamma-irradiation.// *Tsitol Genet.* 2010 Sep-Oct;44(5):18-22. Russian.

 **Alexander G. Akishev, Danila A. Gonchar, Murat A. Abdurashitov and Sergey Kh. Degtyarev Epigenetic typing of human cancer cell lines by BslI- and Glal-PCR assays** // "Ovchinnikov bulletin of biotechnology and physical and chemical biology" V.7, No 3, pp 5-16, 2011

F. Syeda, R.L. Fagan, M. Wean, G.V. Awakumov, J.R. Walker, S. Xue, S. Dhe-Paganon, & C. Brenner, "The RFTS Domain is a DNA-competitive Inhibitor of Dnmt1"//, *JBC*, v. 286, pp. 15344-15351 (2011).

Keith N. Rand, Graeme P. Young, Thu Ho and Peter L. Molloy, "Sensitive and selective amplification of methylated DNA sequences using helper-dependent chain reaction in combination with a methylationdependent



DATA SHEET

[restriction enzymes.](#)//, Nucleic Acids Research, pp. 1-10 (2012).

GENTAUR