



DHN-Substrates: New Chromogens for use in Liquid Media

Glycosynth is delighted to introduce our new range of chromogenic substrates for detecting enzyme activity in liquid media. The new, patented*, chromogenic substrates are based on the core molecule 2,3-dihydroxynaphthalene (**DHN**).

DHN-substrates are colourless. However, when cleaved in the presence of an iron salt they produce an intense, highly-visible, purple colour** which thereby demonstrates a remarkable colour contrast between positive and negative bacterial cultures*. The substrates are easy to use in liquid or tube media under both aerobic and anaerobic conditions. Importantly, they also allow good microbial growth.

A further major advantage over other commonly used substrates is that the intense purple colour is able to mask the yellow produced by ONPG cleavage, making DHN-substrates especially suitable for dual chromogenic systems, such as for the detection of *E. coli* and coliforms. Moreover, it has been observed that the sensitivity of DHN-beta-D-glucuronide CHA [our product code 80065] for *E. coli* detection, is higher than the commonly used substrate X-beta-D-glucuronide CHA [our product code 70065].

Advantages of DHN-Substrates

Dark purple colour is easy to observe

No UV light required

Different colour to other substrates used in liquid media (e.g. ONPG)

Colour masks ONP - suitable for dual chromogen tests incorporating ONPG

Non-toxic to bacteria; good growth

Effective under aerobic or anaerobic conditions

Soluble at 1% in water

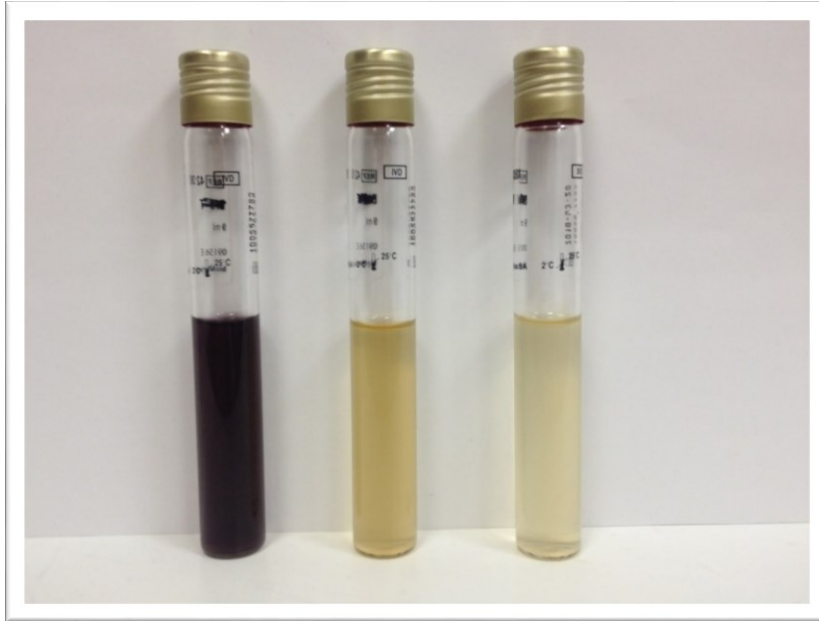
Only requires the presence of an iron salt, as commonly used in X-Gal media

Application

DHN beta-D-ribofuranoside shows the clear advantages offered by these new substrates.

When challenged with three strains of staphylococci, only the *S. aureus* strains NCTC 6571 and MRSA NCTC 11939 were able to hydrolyse it and it was unaffected by *S. epidermidis* NCTC 11047.

This demonstrates one potential application of DHN beta-D-ribofuranoside in the detection of MRSA. DHN beta-D-ribofuranoside is able to differentiate this organism from other species of staphylococci that can cause interference in its positive identification. Other chromogenic beta-D-ribofuranosides have already been evaluated for this purpose [EP1438424].



Above: Detection of beta-ribofuranosidase using DHN beta-ribofuranoside (80180) in *Staphylococcus aureus* NCTC 6571 (left) and lack of reaction shown by *Staphylococcus epidermidis* NCTC 11047 (middle) and *Enterococcus faecalis* NCTC 775 (right).

Microbiological evaluation

	DHN beta-D-glucopyranoside 80059 ^b	DHN beta-D-galactopyranoside 80045 ^b	DHN beta-D-glucuronide CHA salt 80065 ^c	DHN beta-D-ribofuranoside 80180 ^d
Gram-negative microorganisms ^a	Colour	Colour	Colour	Colour
<i>Escherichia coli</i> NCTC 10418	Tr. purple	++ purple	++ purple	++ purple
<i>Serratia marcescens</i> NCTC 10211	++ purple	+ purple	-	++ purple
<i>Pseudomonas aeruginosa</i> NCTC 10662	-	-	-	-
<i>Burkholderia cepacia</i> ATCC 25416	-	-	-	-
<i>Yersinia enterocolitica</i> NCTC 11176	-	-	-	-
<i>Salmonella typhimurium</i> NCTC 74	-	-	-	++ purple
<i>Citrobacter freundii</i> NCTC 9750	NT	NT	-	++ purple ^e
<i>Morganella morganii</i> 462403 (wild)	NT	NT	-	++ purple
<i>Enterobacter cloacae</i> NCTC 11936	+ purple	++ purple	-	++ purple
<i>Providencia rettgeri</i> NCTC 7475	++ purple	-	-	++ purple
<i>Klebsiella pneumoniae</i> NCTC 10896	++ purple	++ purple	NT	NT
<i>Acinetobacter baumannii</i> ATCC 19606	-	-	NT	NT
Gram-positive microorganisms				
<i>Bacillus subtilis</i> NCTC 9372	+ purple	-	-	-
<i>Enterococcus faecalis</i> NCTC 775	+ purple	-	-	-
<i>Enterococcus faecium</i> NCTC 7171	+ purple	Tr. purple	-	-
<i>Staphylococcus epidermidis</i> NCTC 11047	-	-	-	-
<i>Staphylococcus aureus</i> NCTC 6571	-	-	-	+ purple
<i>Staphylococcus aureus</i> (MRSA) NCTC 11939	-	-	-	+ purple
<i>Streptococcus pyogenes</i> NCTC 8306	-	-	-	-
<i>Listeria monocytogenes</i> NCTC 11994	+ purple	-	-	-
Yeasts				
<i>Candida albicans</i> ATCC 90028	-	-	-	-
<i>Candida glabrata</i> NCPF 3943	-	-	-	-

^a NCTC: National Collection of Type Cultures; ATCC: American Type Culture Collection; NCPF: National Collection of Pathogenic Fungi.

^b All microorganisms exhibited moderate growth in the presence of these substrates.

^c The Gram-negative microorganisms exhibited strong growth on the Gram-positive microorganisms and yeasts showed moderate growth in the presence of this substrate.

^d All microorganisms exhibited strong growth in the presence of this substrate.

^e *Citrobacter freundii* 4626 (wild). Diffusion of the colour into the medium was noted in all cases.

++ strong colour, + moderate colour, - no colour, Tr. Trace of colour. NT, not tested.

Pricing

Product Code	Product	Pack size	Price \$	Price €	Price £
80045	DHN beta-D-galactopyranoside 2,3-Dihydroxynaphthalene beta-D-galactopyranoside	500mg	52.00	50.00	40.00
		1g	90.00	83.00	70.00
		2g	148.00	144.00	115.00
80059	DHN beta-D-glucopyranoside 2,3-Dihydroxynaphthalene beta-D-gluopyranoside	500mg	58.00	56.00	45.00
		1g	103.00	100.00	80.00
		2g	155.00	150.00	120.00
80065	DHN beta-D-glucuronide CHA salt 2,3-Dihydroxynaphthalene beta-D-glucuronide cyclohexylammonium salt	250mg	67.00	64.00	45.00
		500mg	113.00	108.00	80.00
		1g	165.00	158.00	120.00
80180	DHN beta-D-ribofuranoside 2,3-Dihydroxynaphthalene beta-D-ribofuranoside	250mg	58.00	56.00	45.00
		500mg	103.00	100.00	80.00
		1g	155.00	150.00	120.00

Related Documentation

[The synthesis of novel chromogenic enzyme substrates for detection of bacterial glycosidases and their applications in diagnostic microbiology.](#) Bioorganic & Medicinal Chemistry 2018, 26 (17), 4841

* [US9938562](#), [EP3066209](#), [US10443084](#)

**The exact colour produced may depend on the other components of the media and can appear as purple or a deep-reddish brown.