

## Nourseothricin

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Additional Information and  
List of Selectable Organisms

## Field of Use

- Streptothricin-class antibiotic for a broad spectrum of bacteria and eukaryotic unicellular or complex organisms (see Table 1)
- Preferred selection antibiotic for genetic modification of:
  - Mammalian cells
  - Yeast and filamentous fungi
  - Protozoa and microalgae
  - Gram-positive and Gram-negative bacteria
  - Plants ... and many more
- Not intended for human consumption
- Also known as clonNAT

## Mechanism of Action

- Antibiotic effect of Nourseothricin through inhibition of protein biosynthesis and induction of miscoding
- Resistance to Nourseothricin conferred by *sat*, *stat* or *nat* marker genes
- Product of the resistance gene – Nourseothricin N-acetyltransferase – inactivates Nourseothricin by monoacetylation of the  $\beta$ -amino group of its  $\beta$ -lysine residue

## Advantages

- Low or no background: Resistance protein is localized intracellularly and cannot be degraded in the cell culture medium
- Not used in human or veterinary medicine, therefore no conflict with regulatory requirements
- No cross-reactivity with other aminoglycosid antibiotics such as Hygromycin or Geneticin
- No cross-resistance with therapeutic antibiotics
- Long-term stable as powder or solution
- Highly soluble in water (1 g/ml)

**Table 1: Organisms suitable for Nourseothricin selection****Mammalian cells**

<b>Cell line</b>	<b>Selection concentration [µg/ml]</b>
HMEC	50
HEK293T	25
BT549	25
U2OS	25
A2780	75

**Yeast**

<b>Species</b>	<b>Selection concentration [µg/ml]</b>
Ashbya gossypii	50-200
Candida albicans	200-450
Candida dubliniensis	100
Candida guilliermondii	150
Candida glabrata	100-200
Candida kefyr	450
Candida lusitanae	100-450
Candida orthopsilosis	200
Candida parapsilosis	200
Candida tropicalis	150-200
Hansenula ciferrii	50
Hansenula polymorpha	100
Kluyveromyces lactis	50-100
Lipomyces starkeyi	30
Pichia ciferrii	50
Pichia pastoris	50-200
Saccharomyces cerevisiae	25-200
Schizosaccharomyces japonicus	50-100
Schizosaccharomyces pombe	40-100
Torulaspora delbrueckii	50
Zygosaccharomyces rouxii	5
Zygosaccharomyces bailii	100

## Other Ascomycota

Species	Selection concentration [µg/ml]
<i>Acremonium chrysogenum</i>	25
<i>Alternaria brassicicola</i>	200
<i>Aspergillus nidulans</i>	120
<i>Aspergillus tubingensis</i>	20
<i>Botrytis cinerea</i>	50-150
<i>Clonostachys rosea</i>	300-400
<i>Coccidioides posadasii</i>	100
<i>Cochliobolus heterostrophus</i>	120-300
<i>Cochliobolus luttrellii</i>	60-100
<i>Colletotrichum coccodes</i>	100
<i>Colletotrichum graminicola</i>	100-400
<i>Colletotrichum higginsianum</i>	100
<i>Cryphonectria parasitica</i>	100
<i>Fusarium fujikuroi</i>	100
<i>Fusarium graminearium</i>	25-200
<i>Fusarium oxysporum</i>	50-60
<i>Leptosphaeria maculans</i>	50
<i>Neurospora crassa</i>	20-200
<i>Penicillium chrysogenum</i>	150-200
<i>Penicillium roqueforti</i>	40
<i>Plectosphaerella cucumerina</i>	100
<i>Podospora anserina</i>	50-75
<i>Rhynchosporium commune</i>	1
<i>Sclerotinia sclerotiorum</i>	40-200
<i>Sordaria macrospora</i>	50
<i>Trichoderma atroviride</i>	300-400
<i>Trichophyton mentagrophytes</i>	50
<i>Verticillium dahliae</i>	50
<i>Yarrowia lipolytica</i>	250
<i>Zymoseptoria tritici</i>	40-50

## Streptomycetes

Species	Selection concentration [µg/ml]
<i>Streptomyces lividans</i>	6-100

## Basidiomycota

Species	Selection concentration [µg/ml]
<i>Cryptococcus gattii</i>	100
<i>Cryptococcus neoformans</i>	100-200
<i>Physisporinus vitreus</i>	110
<i>Rhodosporeidium kratochvilovae</i>	200
<i>Rhodotorula graminis</i>	200
<i>Schizophyllum commune</i>	3-20
<i>Ustilago maydis</i>	75-150
<i>Xanthophyllumyces dendrorhous</i> 6)	30

## Protozoa

Species	Selection concentration [µg/ml]
<i>Crithidia bombi</i>	200
<i>Leptomonas seymouri</i>	250
<i>Leishmania amazonensis</i>	50
<i>Leishmania braziliensis</i>	50-100
<i>Leishmania donovani</i>	50-125
<i>Leishmania infantum</i>	20-100
<i>Leishmania major</i>	30-100
<i>Leishmania mexicana</i>	25-50
<i>Leishmania tarentolae</i>	50-100
<i>Phytomonas serpens</i>	100
<i>Plasmodium falciparum</i>	75
<i>Toxoplasma gondii</i>	500
<i>Trypanosoma brucei</i>	150-200
<i>Trypanosoma vivax</i>	1

## Cyanobacteria

Species	Selection concentration [µg/ml]
<i>Synechocystis</i> sp. PCC 6803	50

## Microalgae

Species	Selection concentration [µg/ml]
<i>Amphora coffeaeformis</i>	300
<i>Chaetoceros</i> sp.	100-500
<i>Chaetoceros gracilis</i>	300-400
<i>Ostreococcus tauri</i>	1500
<i>Phaeodactylum tricornutum</i>	50-250
<i>Thalassiosira pseudonana</i>	100-200

## Plants

Species	Selection concentration [µg/ml]
<i>Arabidopsis thaliana</i>	20-200
<i>Daucus carota</i>	100
<i>Lotus corniculatus</i>	50
<i>Nicotiana tabacum</i>	100
<i>Oryza sativa</i>	20-200

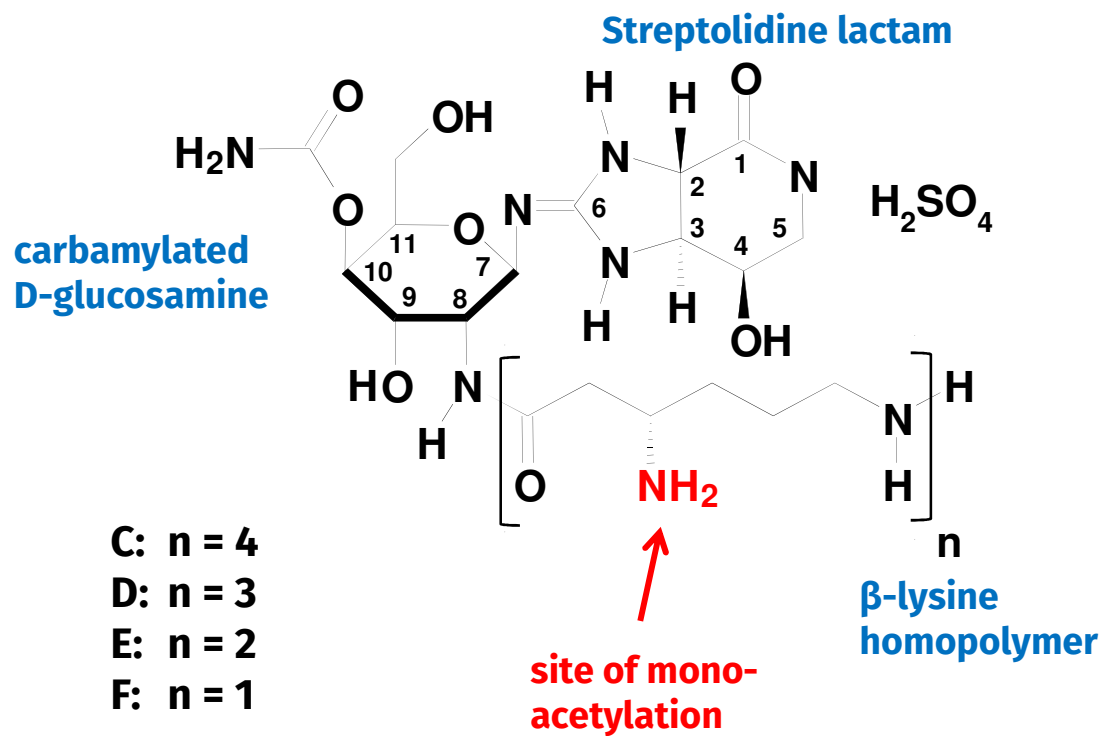
## Gram(-) Bacteria

Species	Selection concentration [µg/ml]
<i>Agrobacterium tumefaciens</i>	100
<i>Escherichia coli</i>	2-50
<i>Francisella tularensis</i>	50
<i>Pseudomonas aeruginosa</i>	50-100

## Gram(+) bacteria

Species	Selection concentration [µg/ml]
<i>Bacillus subtilis</i>	5-50
<i>Enterococcus faecium</i>	8-500
<i>Mycobacterium smegmatis</i>	25
<i>Staphylococcus aureus</i>	2-50

## Nourseothricin structure

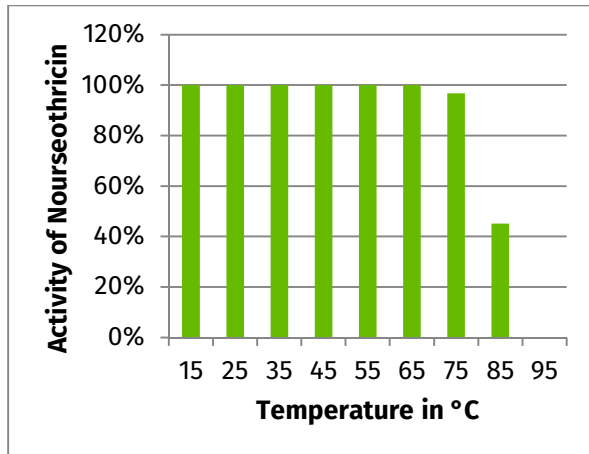


**Figure 1:** Nourseothricin is a natural mixture of streptothricins C, D, E and F produced by *Streptomyces noursei*. It consists of > 85 % streptothricin D+F.

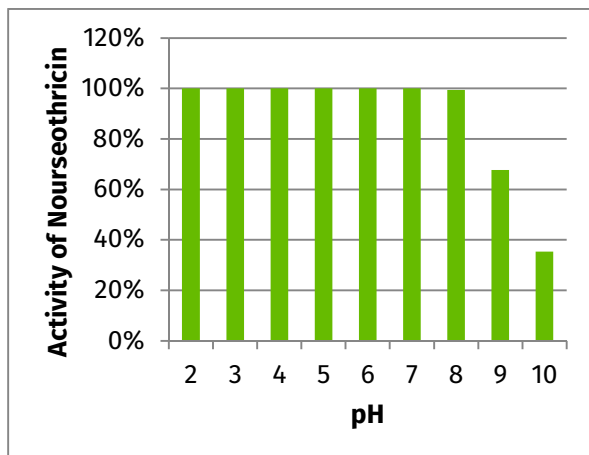
- Molecular weight: ca. 503 g/mol
- CAS#: 96736-11-7
- Appearance: beige powder

## Nourseothricin stability

- Long-term stable without loss of activity
- Powder can be stored for 10 years at 4 °C or for two years at 20°C
- Solution (100 mg/ml) is stable for >24 months at -20 °C or 12 months at 4 °C



**Figure 2:** Nourseothricin solutions are stable at temperatures up to 75 °C even after 24 h of heat treatment. Stock solutions were heated for 24 h at the indicated temperatures and then added at 100 µg/ml concentration to a test culture (organism: *Leishmania tarentolae*). Activity was measured 3 days after incubation.



**Figure 3:** Nourseothricin solutions are stable at pH 2-8 for >7 days at 26 °C. Stock solutions were incubated at indicated pH for 4 days and 26 °C before addition at 100 µg/ml concentration to a test culture (organism: *Leishmania tarentolae*). Activity was measured 3 days after incubation.



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