EXPRESSION OF STABLE ISOTOPE LABELLED PROTEIN WITH SILANTES RICH GROWTH MEDIA FOR **YEAST OD 1 - 5**

Silantes OD media are rich growth media for the expression of stable isotope labelled protein. The media are available in all combinations of the stable isotopes ^{2}H, ^{13}C and ^{15}N. They are formulated for increasing cell densities (optical densities measured at 600nm) containing hydrolysates with increasing concentrations. These OD-values are determined with common yeast stains such as *P.pastoris*.

The following instructions are applicable for

OD 1 **OD 2** **OD 4** **OD 5** OD 10

The Silantes OD1-5 media are bacterial hydrolysates adjusted to M9 conditions. The glucose content is less than 30mg/L.

Silantes OD1 media reach a cell density comparable to 0,2% glucose-M9 media.

Silantes OD2 media reach a cell density comparable to 0,4% glucose-M9 media.
Silantes OD2 media are commonly used if inducing the cells at OD0,6-0,9 followed by a ~4h incubation. The heterologous protein expression performance is similar as with LB medium.

Silantes OD4-, OD5- and OD10 (concentrate)-media are available on request.

The media are available as powder or as ready-to-use Silantes OD media solutions. If using the powder media, proceed with the "**Operating instructions for the preparation of Silantes OD Media Powder for Yeast**". If using the ready-to-use media solution, proceed with the "**Operating instructions for Silantes OD Media Solutions for Yeast**". Please find both operating instructions on page 2.

This product is for laboratory use only. The safety and efficacy of this product in diagnostic or other clinical uses is not established.

Operating instructions for the preparation of Silantes OD Media Powder for Yeast

Following instructions are applicable to obtain 1L of Silantes OD Media Solution. In order to avoid isotopic dilution, use deuterated media powder (which is lyophilized from D_2O-buffer) when preparing the media solution in D_2O.

1. *Prepare 1000mL of H_2O / D_2O.*
2. *Add 10g Silantes Powder Media for Yeast.*
3. *Dissolve the powder.*
   The final yield cell density can be increased by 10-20% by adding a salt mix at this stage.
4. *Sterile filtrate the solution in an appropriate shaker bottle.*

Salt mix and D_2O can be purchased from Silantes.

After preparing the Silantes OD media solution for Yeast, proceed as follows:

Operating instructions for Silantes OD1-5 Media Solutions for Yeast

In order to avoid isotopic dilution by inoculation use an isotopically labelled Silantes OD-media as a preculture.

5. *Fill Silantes OD into a sterile culture flask or fermenter.*
   For optimal aeration, fill your flask to a maximum of 1/3 of the flask volume.
6. *If applicable for your system, add antibiotics and special nutrients.*
7. *Inoculate the Yeast culture.*
8. *Grow the Yeast culture in an orbital shaker or fermenter until the desired optical density is reached.*
   Ensure sufficient aeration. For optimal aeration, Silantes suggests to use an orbital shaker apparatus at > 150 rpm.
9. *Induce the expression of your protein in the usual way.*
10. *Collect the cells.*