

This document covers operation instructions for:

1. The preparation of Silantes OD Solution Medium from Silantes Concentrate Medium,
2. The use of Silantes OD Solution Medium
3. The preparation and use of Silantes SILEX and ECO Powder Medium as a supplement to M9 medium,
4. The preparation and use of Silantes SILEX and ECO Powder Medium as a stand-alone medium,

The following instructions are examples for the use of Silantes cell growth media for *E. coli*. The user may have to modify the procedures depending on the transformed bacterial cells used, based on growth parameters, antibiotic selection, induction, etc.

Following instructions assume the use of D₂O and ¹³C-glucose in some steps. These chemicals can also be obtained from Silantes at sales@silantes.com.

1. The preparation of Silantes OD Solution Medium from Silantes Concentrate Medium

This instruction describes how to prepare the Concentrate Medium to obtain rich growth media yielding the desired optical density.

1. Prepare 10 mL of MgSO₄ solution in H₂O / D₂O as follows:
Dissolve 1.479 g MgSO₄ • 7H₂O / 7D₂O in 10 mL of H₂O / D₂O.
2. Prepare 50 mL of M9 salt solution as follows:
Dissolve 2.125 g of Na₂HPO₄ • 2H₂O / 2D₂O and 0.75 g of KH₂PO₄ in 50 mL H₂O / D₂O.
3. Mix the ingredients depending on your desired cell density as follows:

Desired Cell Density (λ=600nm)	OD2	OD3	OD4	OD5
Silantes Concentrate Medium [mL]	200	300	400	500
M9 salt solution [mL]	50	50	50	50
MgSO ₄ solution [mL]	10	10	10	10
H ₂ O / D ₂ O [mL]	740	640	540	440
Silantes OD Solution Medium [mL]	1000	1000	1000	1000

The glucose content in 1000 mL Silantes OD2 Solution Medium is less than 30 mg.

4. Sterile filtrate the solution and proceed with the fermentation procedure described in paragraph 2.

2. The use of Silantes OD Solution Medium

The Silantes OD2 medium is a ready-to-use rich growth solution medium for a high-performance expression of stable isotope labelled proteins.

1. Fill the sterile Silantes OD Solution Medium into a sterile culture flask or fermenter.
For optimal aeration, fill your flask to a maximum of 1/3 of the flask volume.
2. If applicable for your system, add antibiotics and special nutrients.
3. Inoculate the *E. coli* culture.
4. Grow the culture in an orbital shaker or fermenter until the desired optical density is reached.
Ensure sufficient aeration. For optimal aeration, Silantes suggests using an orbital shaker apparatus at > 150 rpm.
5. Induce the expression of your protein in the usual way. (Commonly at OD₆₀₀0.6 – OD₆₀₀0.9 followed by a ~4 h incubation).
6. Collect the cells.

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

3. The preparation and use of Silantes SILEX and ECO Powder Medium as a supplement to M9 medium

This instruction describes how to prepare and use the SILEX or ECO Powder Medium to supplement M9 experiments to fortify cell growth and protein expression rates.

1. Prepare 1000 mL M9 minimal medium in H₂O / D₂O including 2 g/L glucose. For ²H and/or ¹³C-labelled experiments, use ²H and/or ¹³C-glucose to avoid isotopic dilution.
2. If applicable for your system, add antibiotics and special nutrients.
3. Dissolve 1 g/L powder medium in the M9 medium solution as follows:
 - a. Dissolve 1 g Silantes SILEX or ECO Powder in 10 mL H₂O / D₂O.
 - b. Add the SILEX or ECO Powder solution to the M9 medium.

To avoid isotopic dilution, use deuterated medium powder when preparing the medium solution in D₂O.

4. At this stage, the final yield cell density can be increased by optimizing the MgSO₄ concentration.
5. Sterile filtrate the solution in an appropriate shaker bottle.
6. Inoculate the *E. coli* culture.
7. Grow the culture in an orbital shaker or fermenter until the desired optical density is reached. Ensure sufficient aeration. For optimal aeration, Silantes suggests using an orbital shaker apparatus at > 150 rpm.
8. Induce the expression of your protein in the usual way. (Commonly at OD₆₀₀0.6 – OD₆₀₀0.9 followed by a ~4 h incubation).
9. Collect the cells.

4. The preparation and use of Silantes SILEX and ECO Powder Medium as a stand-alone medium

This instruction describes how to prepare the SILEX or ECO Powder Medium to use it as a stand-alone medium.

1. Dissolve 10 g/L Silantes SILEX or ECO Powder in 800 mL H₂O / D₂O.
2. Add 10 mL of MgSO₄ solution in H₂O / D₂O as follows:

Dissolve 1.479 g MgSO₄ • 7H₂O / 7D₂O in 10 mL of H₂O / D₂O and add it to the solution.
3. Add 50 mL of M9 salt solution as follows:

Dissolve 2.125 g of Na₂HPO₄ • 2H₂O / 2D₂O and 0.75 g of KH₂PO₄ in 50 mL H₂O / D₂O and add it to the solution.
4. Fill the solution up to 1000 mL with H₂O / D₂O.
5. If applicable for your system, add antibiotics and special nutrients.
6. Sterile filtrate the solution in an appropriate shaker bottle.
7. Inoculate the *E. coli* culture.
8. Grow the culture in an orbital shaker or fermenter until the desired optical density is reached. Ensure sufficient aeration. For optimal aeration, Silantes suggests using an orbital shaker apparatus at > 150 rpm.
9. Induce the expression of your protein in the usual way. (Commonly at OD₆₀₀0.6 – OD₆₀₀0.9 followed by a ~4 h incubation).
10. Collect the cells.

The glucose content in 1000 mL stand-alone medium is less than 30 mg.

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