



One New Hampshire Avenue
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nBIONIX Hypoxic Cell Culture Kit

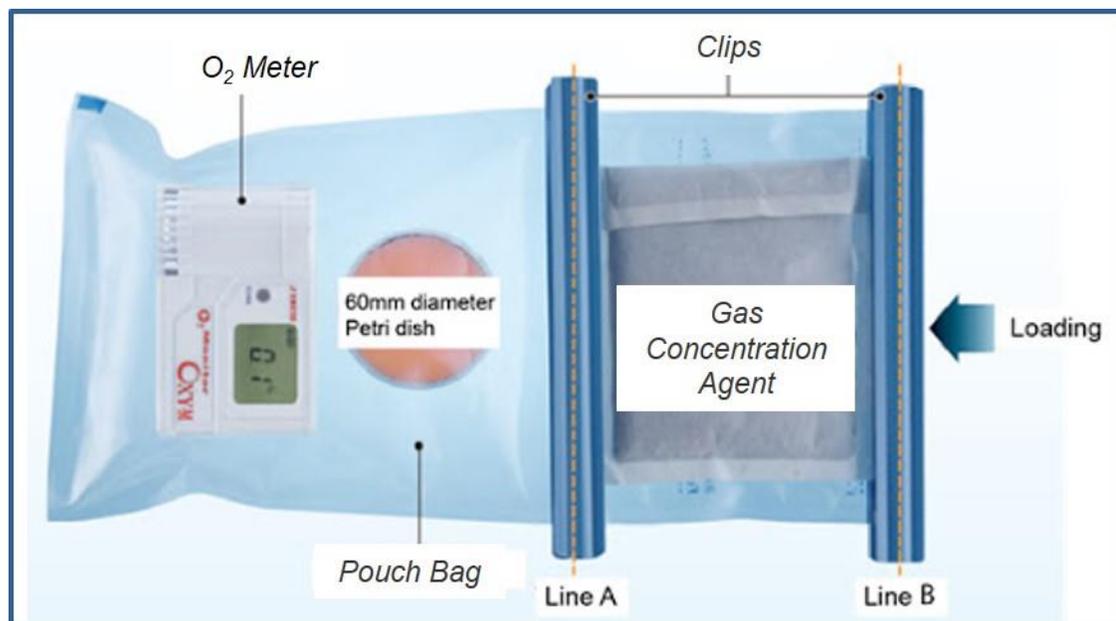
(Part# nBIONOX-1, nBIONOX-2, nBIONOX-3)

Contents: O₂ Meter, 2x pouch Clips, 20x Pouch Bags, 20x packets of Gas Concentration Agent

This Hypoxic Cell Culture Kit enables hypoxic (low oxygen) growth conditions for cells in culture. This simple and convenient kit utilizes a gas barrier pouch bag and gas concentration agent (O₂ scavenger) allowing a user the desired control of O₂ concentration between 0.1-15% for desired cell growth conditions.

Directions:

- 1) Place the O₂ meter into the back of the gas barrier *Pouch Bag*. Place the petri dish or plate filled with culture medium in front of the O₂ meter.
- 2) Open the aluminum bag and take out the white packet containing the *gas concentration agent* (O₂ scavenger). Place it in front of the petri dish/plate*Please refer to the picture below.
- 3) Close the mouth of the bag (*Line B*) with the clip. O₂ inside of the pouch will be absorbed in about 10 minutes providing a hypoxic environment. Please provide a space between the petri dish and the gas concentration agent when closing the clip, so that the second clip may be placed later. If there is not enough room, then fold the packet containing the gas concentration agent.
- 4) Monitor the O₂ meter. When it reaches the desired O₂ concentration, place the second clip onto *Line A* to stop the O₂ absorption and set the oxygen level for the pouch chamber containing the cells and O₂ meter. Please see image below and be careful not to wrinkle the pouch bag at this line.
- 5) Depending on the desired oxygen concentration and the number of the petri dishes/plates to be put in, it may be necessary to optimize the initial starting concentration. **Seal with the second clip (*Line B*) when the oxygen concentration is about 0.1-0.3% lower than the desired oxygen concentration.** In this case, it will approach the desired oxygen concentration over time.





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Notes and required operations

How to use *Clip*: Carefully take care to seal the *Pouch Bag* on a hard, flat surface by applying weight while holding it by hand from the end. When removing the *Clip*, grasp the upper and lower edges with both hands and pull strongly. Be careful that the petri dish is not tilted and the culture medium does not spill. When closing with *Clip*, be careful not to wrinkle as the gas barrier pouch bag will not be airtight if wrinkled.

Tips to loosen *Clip*: Sometimes new *Clip* can be too tight and are difficult to open and close. You can “break in” the *Clips* by first relaxing the plastic. Seal *Clips* over two gas barrier *Pouch Bags* in advance and close it for 2-3 days to loosen it slightly. It is also effective to put it in an incubator for further loosening.

How to handle the *Gas Concentration Agent*: Oxygen absorption begins as soon as the aluminum bag opens. Therefore, prepare the experimental set-up first, before opening the aluminum bag. Open the aluminum bag and immediately place the *Gas Concentration Agent* in the *Pouch Bag* and quickly close it with *Clip*. The *Gas Concentration Agent* generates heat and produces water vapor, indicating that it is reacting. The generated heat subsides immediately and does not affect the culture.

Tips for using the *O₂ Meter*: You can leave the *O₂ Meter* battery in. When using for the first time, please push the middle CAL button (black button) of *O₂ Meter*. The displayed reading should be between 20.6% and 20.8% after the calibration is performed. After about 10-30 minutes (the time varies depending on the number of the petri dishes), the oxygen concentration will decrease to about 5%. After that, it will decrease more slowly.

Tips for controlling the oxygen concentration: Because of limited diffusion the gas inside the petri dish takes some time to exchange with the gas inside the *Pouch Bag*. For example, when the *O₂ Meter* first displays the oxygen concentration in the gas barrier *Pouch Bag* to be ~5%, the oxygen concentration inside the petri dish is slightly higher (~7% to 9%). The oxygen inside the gas barrier *Pouch Bag* slowly diffuse out of the petri dish over time, so the oxygen concentration in the plastic bag may rise a little over this time. If the desired oxygen concentration is as high as 5-10%, there will be no appreciable fluctuations. But if the desired oxygen concentration is 0.5% to 1.0%, then the fluctuation will be larger. To prevent this for ultra-low hypoxic conditions, when the oxygen concentration approaches the desired concentration, grab the lid of the petri dish from above the gas barrier pouch bag and move the lid up and down to replace the air inside.

In the case that the final oxygen levels are required to be tightly controlled ($\pm 0.1\%$) even after an overnight incubation, it is necessary to store the culture medium and *O₂ meter* in anoxic (*O₂ free*) condition in advance. Otherwise, the oxygen dissolved in the culture medium and found within the *O₂ meter* may release after an extended period.

Disposal of used *Gas Concentration Agent*: The *Gas Concentration Agent* may be active after use. Leave it on the desk for about one hour and ensure that there is no residual heat generations before discarding it.

Storing the *Gas Concentration Agent*: *Gas Concentration Agent* should be refrigerated in sealed aluminum packets for long-term storage.

*All equipment except the *Gas Concentration Agent* can be reused.