
# HypoxyCOOL™

Accelerated Low Oxygen Media Conditioning System

BAKER RUSKINN

# Accelerated Oxygen Conditioning for Tissue/Cell Culture Media

HypoxyCOOL™ (patent pending) is a protocol-driven and tested solution that helps improve research results by eliminating media conditioning errors. HypoxyCOOL quickly and precisely reduces media oxygen concentration to target values while maintaining media sterility, temperature and pH. When used in conjunction with a hypoxia workstation, HypoxyCOOL is part of a complete oxygen-regulated, closed-culture system. HypoxyCOOL helps you:

- Improve research results – Avoid abnormal cell interactions and increase cell viability by simulating in vivo-like oxygen conditions directly in the growth media.
- Enhance experimental control – Protocol-driven, tested solution helps eliminate media conditioning errors and preserves media integrity.
- Increase productivity – Fast and efficient conditioning process reduces oxygen concentration to 2% within three hours. Conditioned oxygen level stays low for up to 21 days, when refrigerated.


For stem cell expansion or any tissue culture process requiring accurate and stable oxygen levels

## Choose HypoxyCOOL™

The fundamentals for a good research model not only includes the environment under which cells are contained, but also the media in which cells are grown.

Exposing cells native to low-oxygen (2-8%) environments to normal atmosphere (21% $O_2$) causes abnormal cell interactions and reduces cell viability. Moreover, a controlled environment for achieving and maintaining atmosphere and media conditions within nontoxic pH parameters (7.0-7.4) is crucial for cellular-based work. Therefore, gas controlled incubators and controlled atmosphere workstations are often used to simulate normal conditions in the body's organ systems (see figure 1) and provide a better understanding of understanding of some of the complex processes involved in low oxygen culture work.

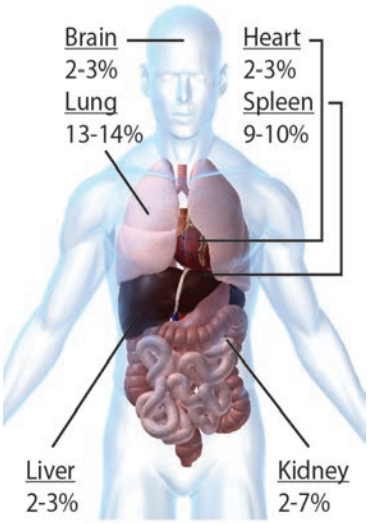


Figure 1

While gas controlled incubators and workstations help to control the atmosphere under which research is performed, cell and tissue cultures still remain prey to oxidative stress; contrasting concentrations of dissolved oxygen between the cells and cell growth media still exists. In fact, new cultured media contains approximately 10-12% dissolved oxygen content by mass, whereas certain tissue cells are typically plated best at 1-3% oxygen, depending upon the research application.

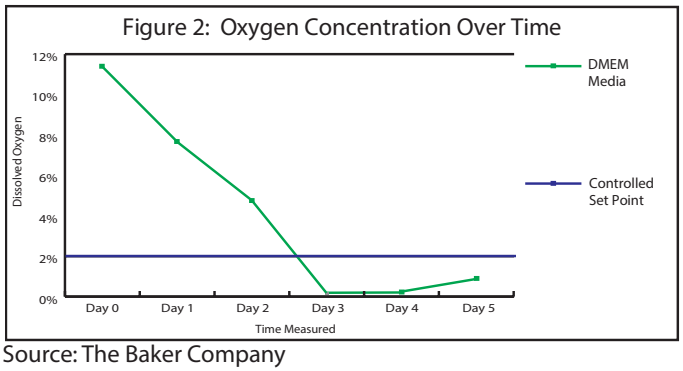
To combat this, most researchers are performing their own methods to condition media to a desired level of oxygen concentrations for their research application. Little documented research is available that further examines these methods, including the precision and accuracy of the actual dissolved oxygen content within the cultured media.

## Conventional Methods Just Aren't Enough

When hypoxic conditions are required, the typical process for conditioning (i.e., reducing the oxygen concentration in) cell culture media is time-consuming and unpredictable. A controlled-oxygen chamber is set to the desired oxygen level, while media is incubated in the chamber for some time before inoculating cells. There are three problems with this empirical conditioning approach.

- **Imprecise Oxygen Measurement -** Current practices assume that the dissolved oxygen content in the media will eventually equilibrate the to the desired level of oxygen required for the application. Based on Baker Ruskinn's research, while the oxygen setting in the chamber may provide 2% oxygen within the environment of the chamber, the actual level of oxygen within the media is much greater and may not reach that level for a significant period of time.

Figure 2: Oxygen Concentration Over Time



Source: The Baker Company

- **Lost Productivity** – Obtaining true, physiologically relevant oxygen levels in media requires significant time and resources within the laboratory. Figure 2 highlights that - dependent upon the conditions and solutions being utilized – it may take over 2 days to achieve an actual dissolved oxygen concentration of 2% within the media itself. Moreover, this process would need to be repeated each time media conditioning is required.
- **Unpredictable Results** – Baker Ruskinn's research shows that there are a number of variables which may impact the traditional conditioning process. Depending upon the duration of the conditioning process, we found that pH levels within the media varied, was often skewed to an abnormally high alkaline or low acidity reading. More importantly, the media (which is clean, but not sterile) when capped or HEPA filtered in a bench, workstation or chamber may become susceptible to microbial contamination when conditioned for a significant period of time. Additionally, the conditioning process may also be impacted by other variables such as altitude, temperature and gas exchange (carbon dioxide).

Even if media integrity is preserved, tissue culture media conditioned by traditional methods may contain higher-than-expected dissolved oxygen levels. This may cause cellular behavior reflective of adaption to abnormal oxygen concentrations, rather than a study that reflects a physiologically-accurate oxygen level for that cell-based experiment.

# HypoxyCOOL™: A New Approach

HypoxyCOOL™ is the first commercialized solution designed to quickly and precisely condition liquid media from an ambient (or unknown) oxygen concentration to a user-defined oxygen level. HypoxyCOOL helps improve research results, enhance experimental control and, ultimately, increase productivity within the laboratory.

## Enhance Experimental Control

HypoxyCOOL helps improve experimental control by eliminating errors in media conditioning. Many variables impact the rate of deoxygenation of liquid media, including but not limited to the type of media being utilized, the temperature range, and your laboratory's altitude. HypoxyCOOL helps you control these variables and provides a repeatable conditioning protocol that improves the consistency of your results.

HypoxyCOOL also helps preserve the integrity of your media during the conditioning cycle, keeping your cells safe from contaminants and variations in pH. HypoxyCOOL's easily-programmable refrigeration system keeps media cold, while carbon dioxide used during the conditioning cycle keeps media at its manufactured pH.

HypoxyCOOL is adaptable to your particular application and the level of oxygen required. The unit is pre-programmed with a set of standard parameters (oxygen level, temperature, shaker speed, and run time) that can be easily modified with simple touchscreen controls.

## Improve Research Results

HypoxyCOOL™ is a vital step for improving cell yield and reducing artifact-driven gene expression changes in all tissue culture processes. Culture media conditioned with HypoxyCOOL boosts the effects of a conventional tri-gas or $CO_2/O_2$ incubator or controlled-oxygen workstation by providing a physiologically-accurate oxygen level in the immediate cellular environment, further improving your research results.

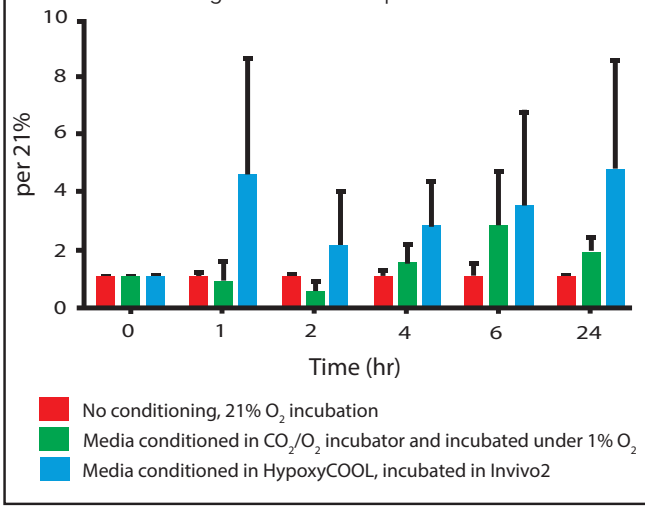
Research conducted in Baker Ruskinn's laboratory and performed independently has shown that using in vivo-like oxygen levels in culture media stabilizes cell cultures, enhances gene expression, while increasing viability and transcriptional stability.

Independent research indicates that HypoxyCOOL provides up to 150% reduction in high-oxygen artifact-driven gene expression changes when HypoxyCOOL is used in conjunction with the Ruskinn SCI-tive hypoxia workstation.

*"VEGF is expressed earlier and to a significantly higher degree when utilizing a HypoxyCOOL / Invivo2 system as opposed to a tri-gas, $CO_2/O_2$ incubator with a set of assays at 1% $O_2$ or ambient $O_2$."*

*- Dr. Clare Yellowley, UC Davis Professor and Researcher*

Figure 3: VEGF Expression




Improved gene expression when media is conditioned with HypoxyCOOL™
Source: Dr. Clare Yellowley and PI Dr. Alice Wong, University of California, Davis

Figure 4: Increase In Cells/Flask with HypoxyCOOL™

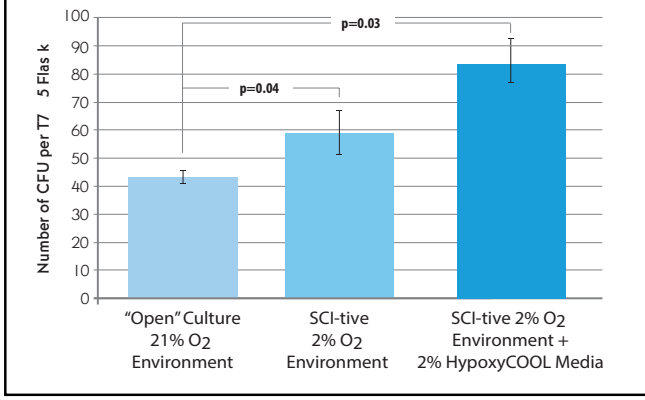
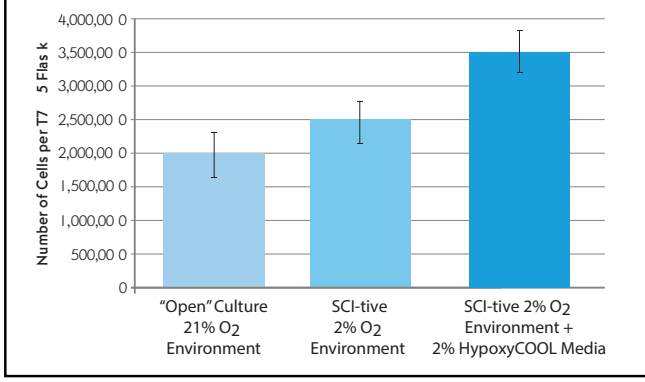



Figure 5: Increase In CFU/Flask with HypoxyCOOL™

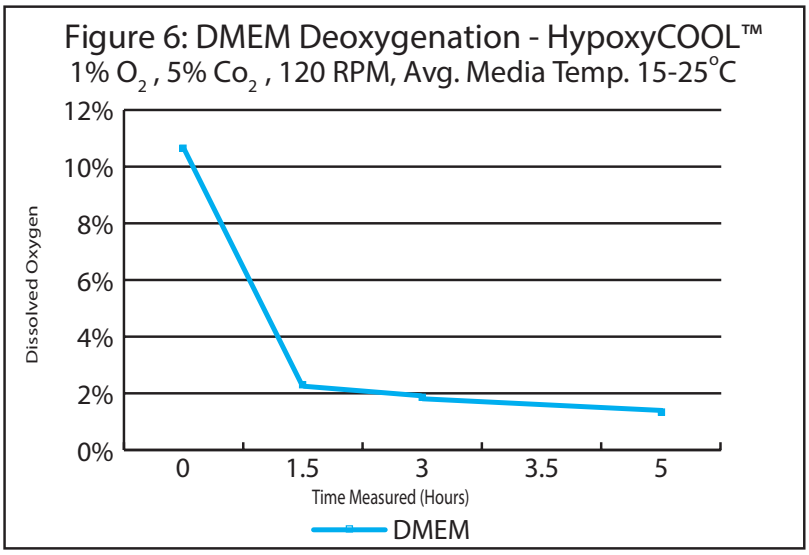



Source: Dr. Nicholas Forsyth Ph.D., Keele University Medical School.

## Increase Your Lab's Productivity

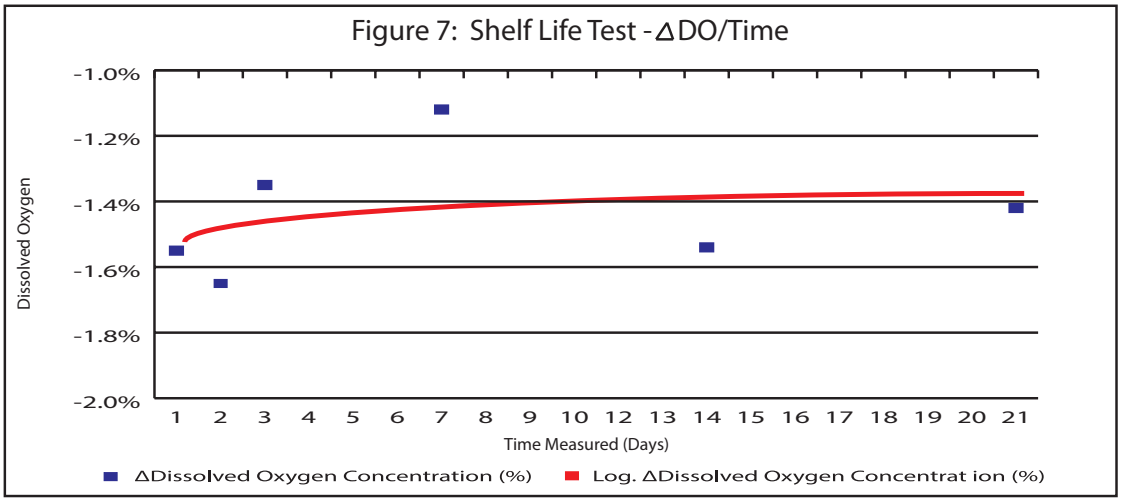
Save time and increase productivity in your lab by switching from a conventional media conditioning method to HypoxyCOOL.

Conditioning your media with HypoxyCOOL significantly reduces the time required to achieve a desired level of oxygen concentration in the media. A standard HypoxyCOOL cycle (1% $O_2$ at 5% $CO_2$ at 120RPM) yields 2% $O_2$ within 2 hours.




Source: The Baker Company

Oxygen levels in media conditioned with HypoxyCOOL stay low for up to 21 days, eliminating the need for re-conditioning.




Source: The Baker Company

HypoxyCOOL is fully compatible with tri-gas incubators and hypoxia workstations, so it easily integrates with your existing laboratory setup.

# The HypoxyCOOL™ Cycle

HypoxyCOOL employs gas conrol, agitation and cooling to aid in efficient gas exchange and media conditioning.

### Gas Mixer

HypoxyCOOL's gas mixing system provides a stabilized atmosphere within the chamber for deoxygenating media. Gas concentration levels are easily programmable, providing consistent, precise and repeatable results. Carbon dioxide is required to help stabilize the process and account for variations in media and media types.

### Refrigeration System

HypoxyCOOL's refrigeration system keeps media cold to ensure it is not compromised during the conditioning cycle. Cooling is required to achieve maximum conditioning results, providing less variation in the conditioning process and maintaining the integrity of the culture media.

### Programmable Orbital Shaker

HypoxyCOOL's inner chamber contains an orbital shaker that agitates media during the conditioning cycle. Agitation aids efficient gas exchange between the chamber atmosphere and the media, speeding the conditioning process.

HypoxyCOOL is easy to use, and offers several user-defined parameters to adapt to a variety of media types.

## Replace Bottle Caps to Keep Media Contaminant-Free

Before starting HypoxyCOOL, replace original media bottle caps with special gas-permeable caps in a sterile manner. Gas-permeable caps contain a small HEPA filter, which keeps media protected from contaminants while allowing oxygen to diffuse from the media bottle head space to the atmosphere of the inner chamber.

## Set Cycle Parameters With Simple Touchscreen Controls

HypoxyCOOL's clear, intuitive touchscreen interface makes it easy to program cycle parameters. Simply select the desired temperature, orbital shaker speed, and the desired oxygen and carbon dioxide concentrations within the chamber. During the cycle, the temperature and gas composition are maintained at the selected set points.

## Remove and Use (or Store) Conditioned Media

After the cycle time elapses, HypoxyCOOL provides an audio and visual indication that the cycle is complete, indicating that the media bottles are ready to be removed. After replacing the gas-permeable bottle caps with the original caps, the conditioned media is now ready for use or storage.

# Technical Specifications

| | | Imperial | Metric |
|---|---|---|---|
| Nominal exterior size | Width | 20.25″ | 514 mm |
| | Depth | 24.75″ | 629 mm |
| | Height | 34.875″ | 886 mm |
| Nominal interior dimensions | Width | 16.125″ | 410 mm |
| | Depth | 15″ | 381 mm |
| | Height | 13.75″ | 349 mm |
| Media tray dimensions | Width | 12″ | 302 mm |
| | Depth | 12″ | 302 mm |
| Weight | | 202 lbs. | 91.6 kg |
| Shipping weight | | 237 lbs. | 107.5 kg |
| Performance | | | |
| Refrigeration range | | 35.6°F - 42.8°F (2°C - 6°C) ; range +/- 2.0° Adjustable in .1° increments | |
| Chamber $O_2$ set point range | | 0.5% – 15.0%; range +/- .1% Adjustable in .1% increments | |
| Chamber $CO_2$ set point range | | 0% - 10.0%; range +/- .1% Adjustable in .1% increments | |
| Conditioning cycle setting range | | 30 minutes – 16 hours | |
| Shaker speed set point range | | 70 rpm – 120 rpm | |
| Gas inlet | | Nitrogen, Carbon Dioxide | |
| Media conditioning capacity | | Nine (9) 500 ml bottles | |
| Construction | | | |
| Refrigeration Chamber | | 18 gauge 304 stainless steel | |
| Internal shaker plates | | Acrylic | |
| Exterior panels | | 18 and 16 gauge carbon steel with powder-coat finish | |
| View window | | Argon-filled double-pane glass | |
| Electrical Requirements | | | |
| Typical motor current | | 4.5 A | |
| Typical operating current | | 6.6 A | |
| Power consumption | | 520 W | |
| Service requirements | | 120V AC, 15 A, 60 Hz | |
| Required circuit protection | | 20 A | |

## Standard Features

- Refrigeration system
- Shaker table
- Media basket with adjustable bottle holders
- USB connection port
- Color touch screen control
- Temperature control
- Oxygen level control
- Carbon dioxide level control
- Power cord
- Gas connection lines for nitrogen and carbon dioxide (2 separate lines and fittings)

## Options & Accessories

- Pack of (12) 500 ml Nalgene media bottles
- Pack of (12) vented Nalgene media bottle caps
- Calibrated dissolved-oxygen bench meter
- Calibrated pH and temperature meter
- Nitrogen gas bottle regulator
- Auto tank switchover module
- Carbon dioxide gas bottle regulator
- Gas tank filter module (one required per regulator)

The Ruskinn brand was founded in 1993 and rapidly became established as one of the world's leading suppliers and manufacturers of anaerobic and modified atmosphere workstations for hypoxia research.

In July 2011, Ruskinn Technology Limited became a wholly-owned subsidiary of The Baker Company Inc., located in Sanford, Maine, USA. Today there are over 1,900 Ruskinn installations in over 40 countries.

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