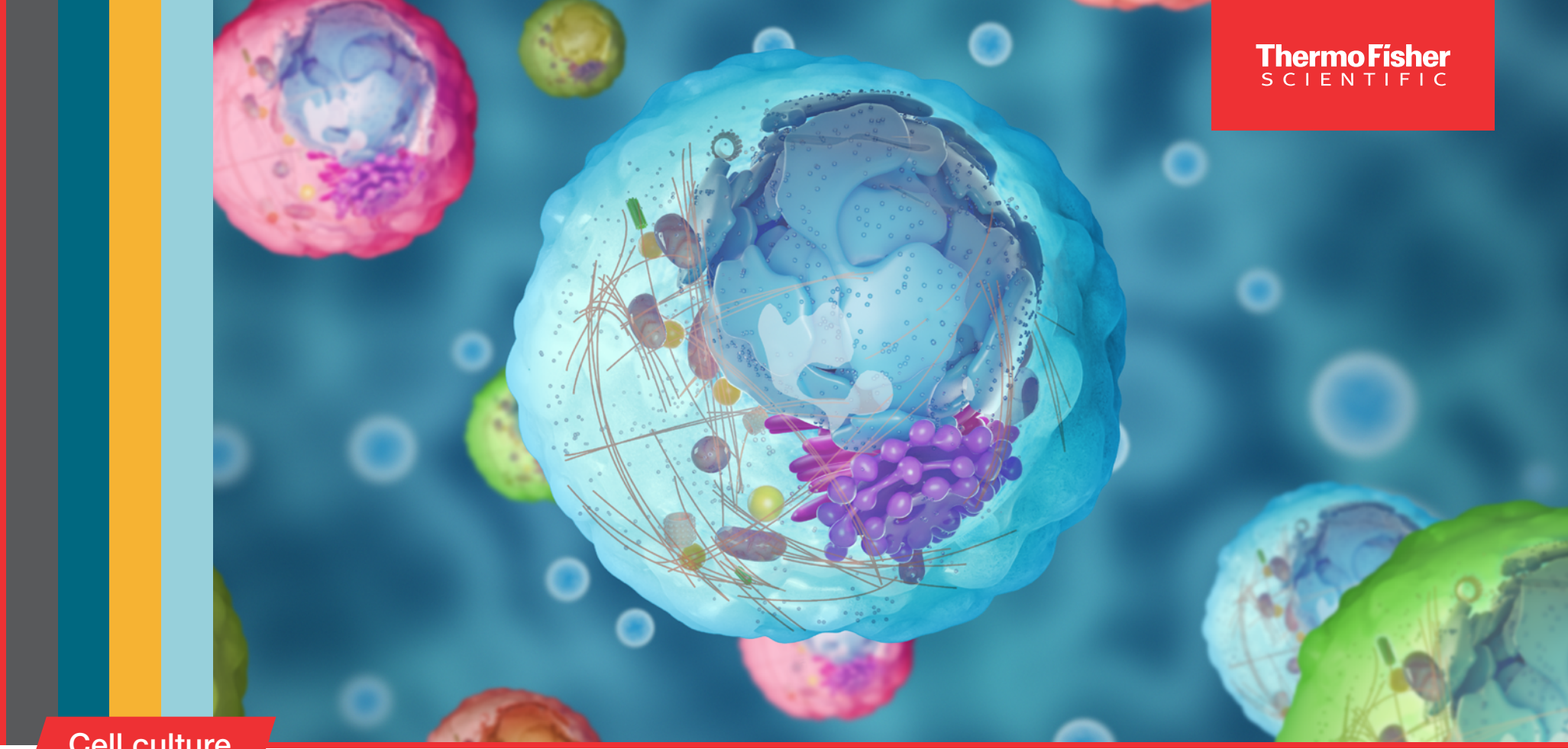


Cell culture

Five reasons

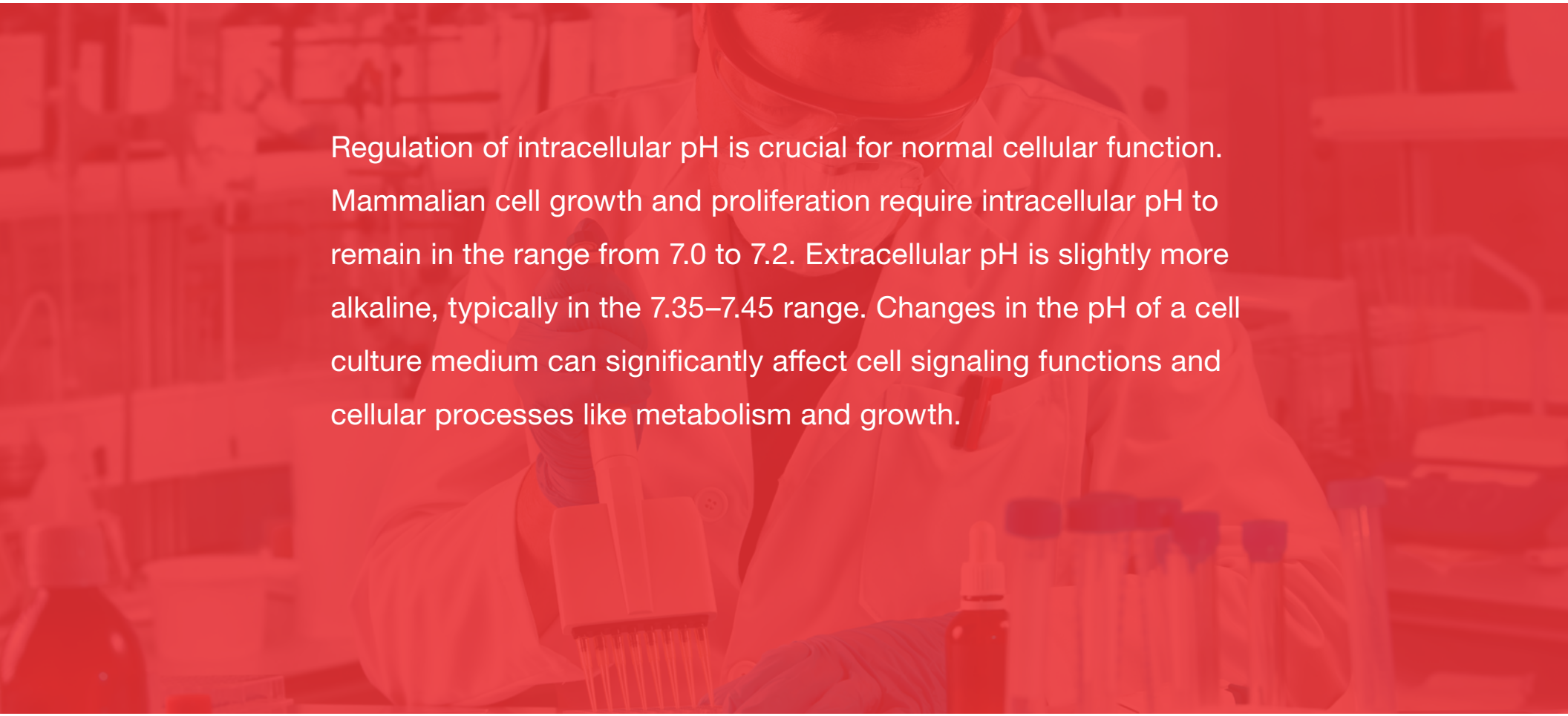
why the right pH is critical for cell health

thermo scientific

In this e-book, we discuss how pH can impact cell health, transfection efficiency, and the production, structure, and behavior of proteins. We also review best practices for accurate pH measurement and describe common problems encountered by researchers and manufacturers. Finally, we offer some suggestions for appropriate selection and care of pH meters and electrodes to help maximize your likelihood of success.

1

Cells are not healthy when the pH is too acidic or too basic.



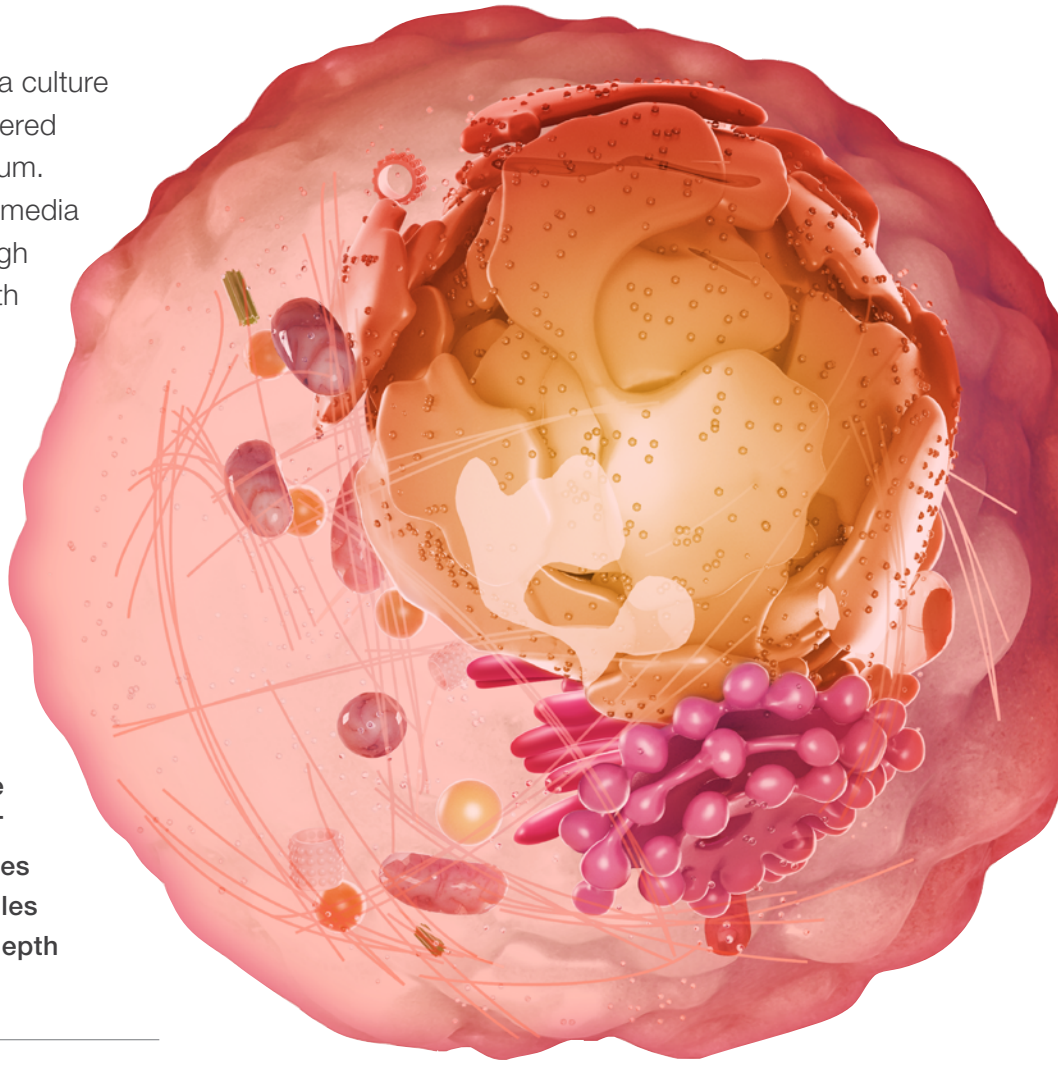
Regulation of intracellular pH is crucial for normal cellular function. Mammalian cell growth and proliferation require intracellular pH to remain in the range from 7.0 to 7.2. Extracellular pH is slightly more alkaline, typically in the 7.35–7.45 range. Changes in the pH of a cell culture medium can significantly affect cell signaling functions and cellular processes like metabolism and growth.

1

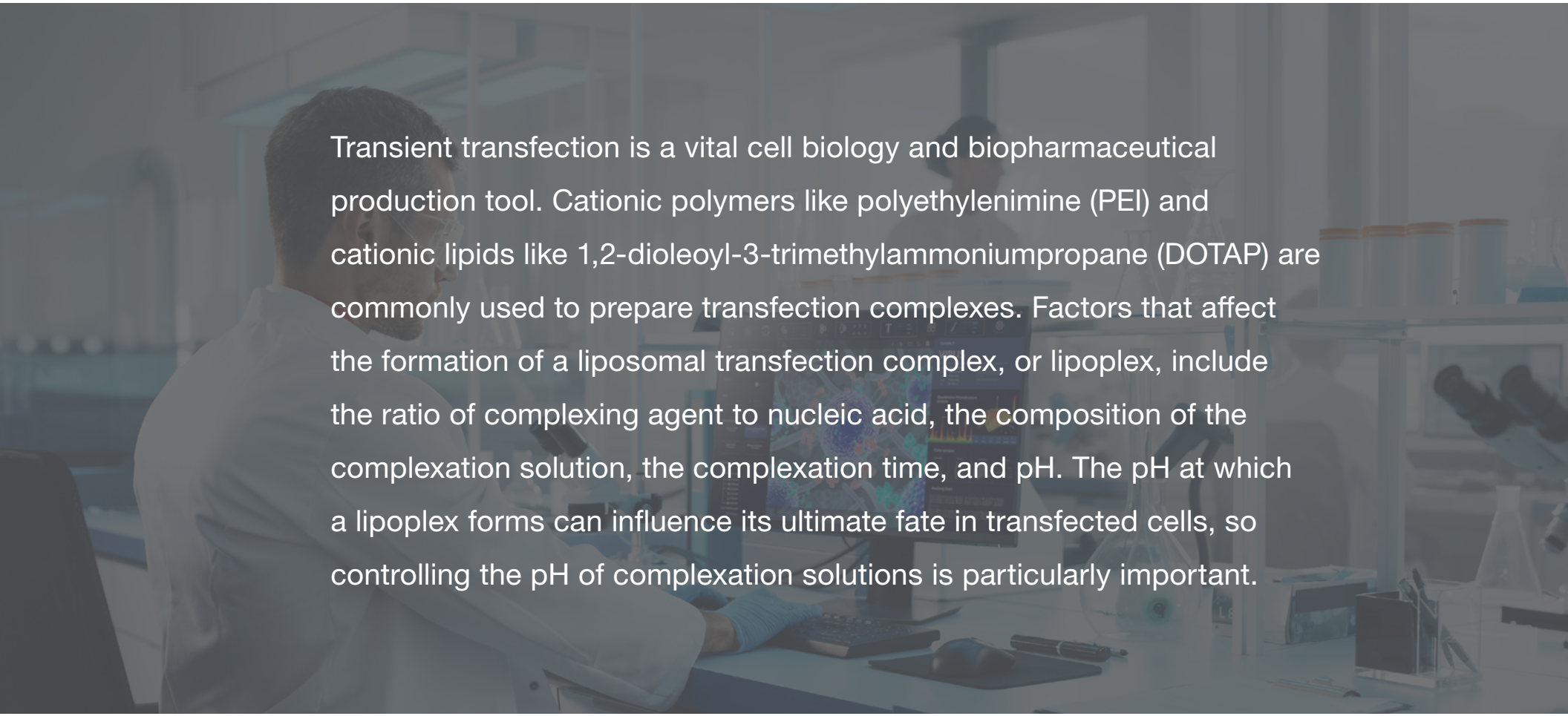
Cells are not healthy when the pH is too acidic or too basic.

Metabolic activity inevitably disturbs the pH of a culture medium, although the extent to which pH is altered depends on the buffering capacity of the medium. Indicator dyes are commonly added to culture media to visually assess pH, but they only enable rough estimation. Indicator dyes can also interfere with spectrophotometric measurements.

To help ensure you obtain high-quality data and take reproducible pH measurements, use the Thermo Scientific™ Orion™ PerpHecT™ ROSS™ Combination pH Micro Electrode to measure the pH of culture media in your laboratory. The Orion PerpHecT ROSS Combination pH Micro Electrode enables researchers to measure the pH of small samples in 384-well plates or samples with a 4.5 mm depth of immersion.



2 The pH of complexation solutions affects transfection efficiency.

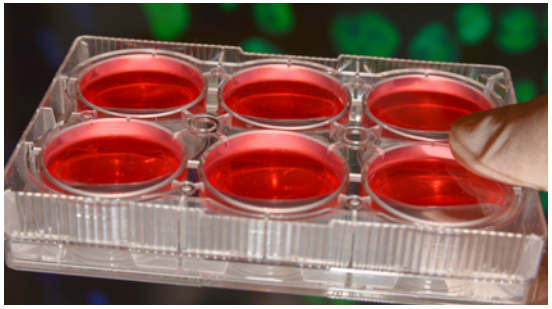


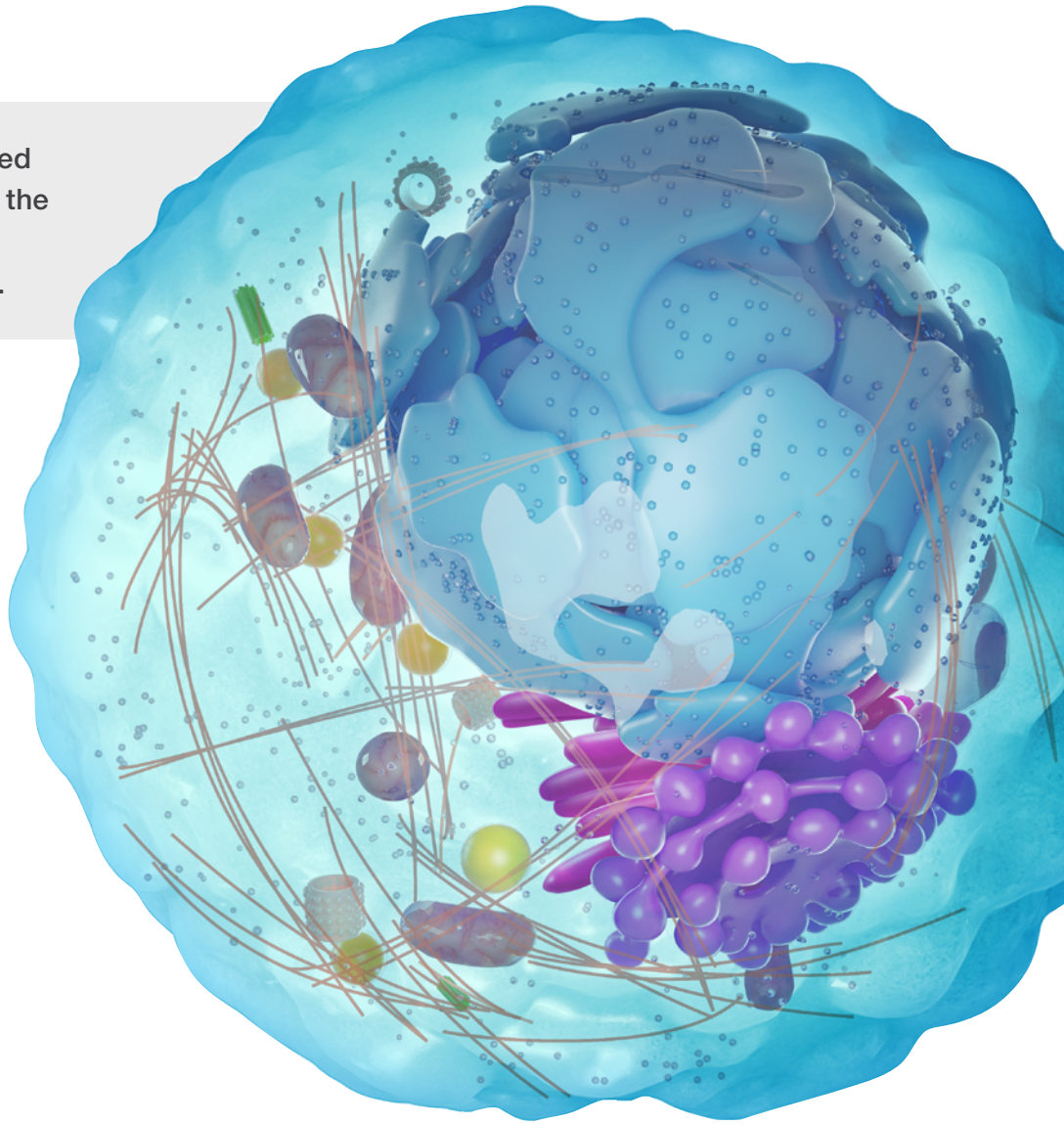
Transient transfection is a vital cell biology and biopharmaceutical production tool. Cationic polymers like polyethylenimine (PEI) and cationic lipids like 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) are commonly used to prepare transfection complexes. Factors that affect the formation of a liposomal transfection complex, or lipoplex, include the ratio of complexing agent to nucleic acid, the composition of the complexation solution, the complexation time, and pH. The pH at which a lipoplex forms can influence its ultimate fate in transfected cells, so controlling the pH of complexation solutions is particularly important.

2

The pH of complexation solutions affects transfection efficiency.

When nucleic acid is complexed with a cationic lipid, the pH of the complexation solution clearly affects transfection efficiency.

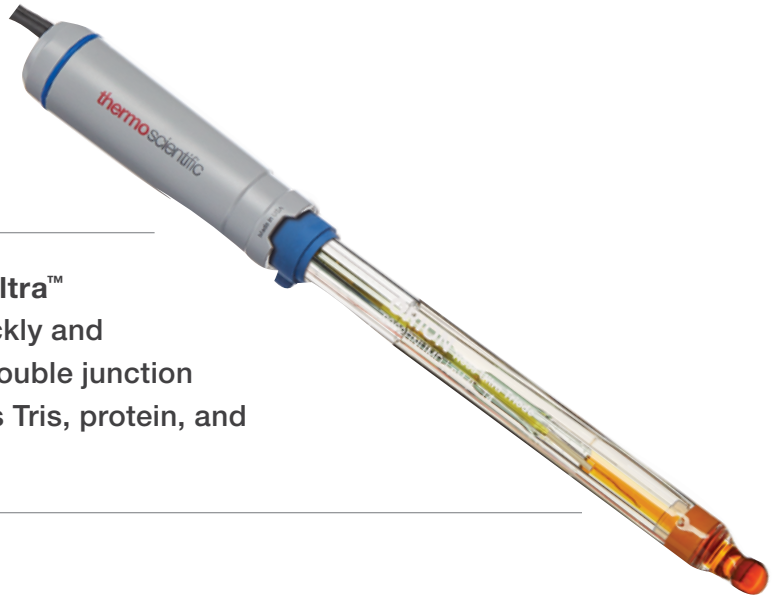




2

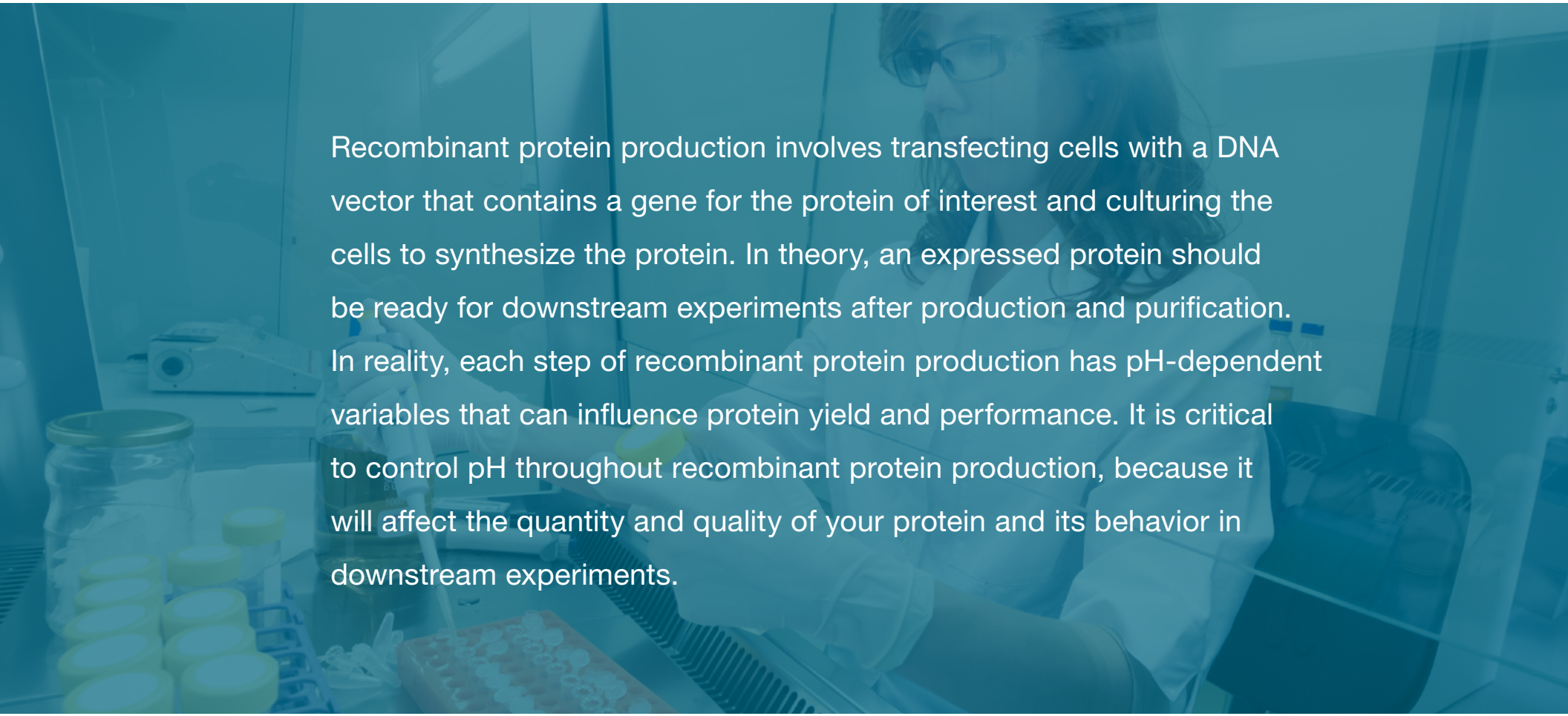
The pH of complexation solutions affects transfection efficiency.

Transfection efficiency will drop if the complexation solution is too acidic, which is likely because the positive charge on the lipoplexes is too high. The cationic lipid may bind too tightly to the negatively charged nucleic acid and inhibit its release from the lipoplexes once they are inside the cell. As the complexation solution becomes more basic, transfection efficiency will increase. This is likely because weakly charged lipoplexes decomplex more readily in the cytosol and release the nucleic acid for uptake into the nucleus (DNA) or translation on ribosomes (mRNA).



Choose a Thermo Scientific™ Orion™ ROSS Ultra™ Triode™ pH/ATC Electrode that responds quickly and provides a stable, accurate pH reading. The double junction design protects your sample from silver and is Tris, protein, and sulfide compatible.

3 Solutions must be at the proper pH when analyzing recombinant proteins.



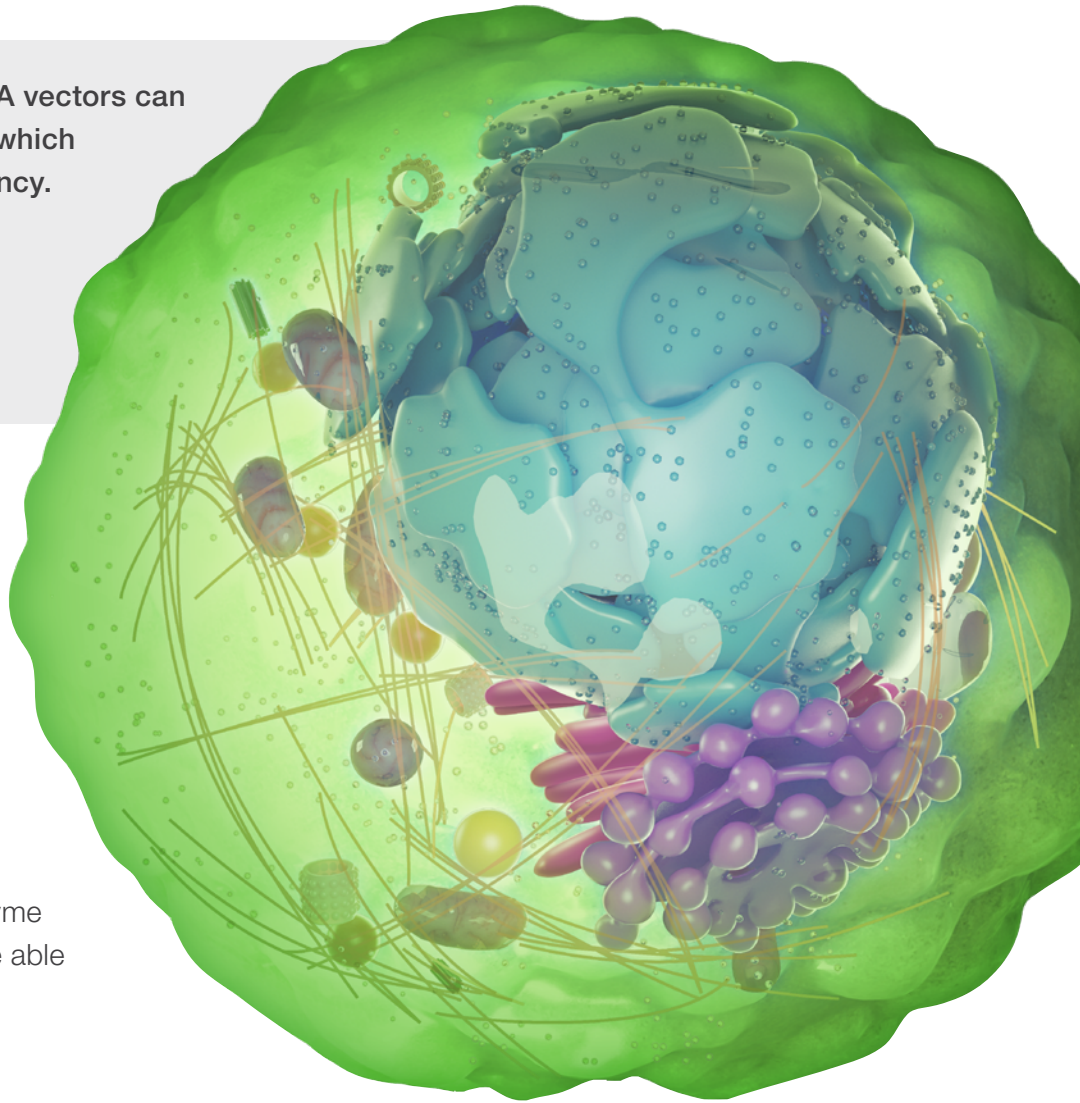
Recombinant protein production involves transfecting cells with a DNA vector that contains a gene for the protein of interest and culturing the cells to synthesize the protein. In theory, an expressed protein should be ready for downstream experiments after production and purification. In reality, each step of recombinant protein production has pH-dependent variables that can influence protein yield and performance. It is critical to control pH throughout recombinant protein production, because it will affect the quantity and quality of your protein and its behavior in downstream experiments.

3

Solutions must be at the proper pH when analyzing recombinant proteins.

Improperly stored plasmid DNA vectors can degrade prior to transfection, which will reduce transfection efficiency. To prevent degradation, DNA vectors used for recombinant protein production should be stored in TE buffer at pH 8.0.

Individual amino acids in a folded protein are exposed to different local environments, and a protein may fold improperly if its isoelectric point (pI) and the pH are misaligned. Misfolding can destabilize or over-stabilize a recombinant protein and lead to functional collapse, as a misfolded protein cannot fully flex its structure to interact with its intended target. Enzymes are particularly susceptible to pH variations in local environments, and an enzyme stored or purified at the wrong pH will not be able to properly catalyze the desired reaction.

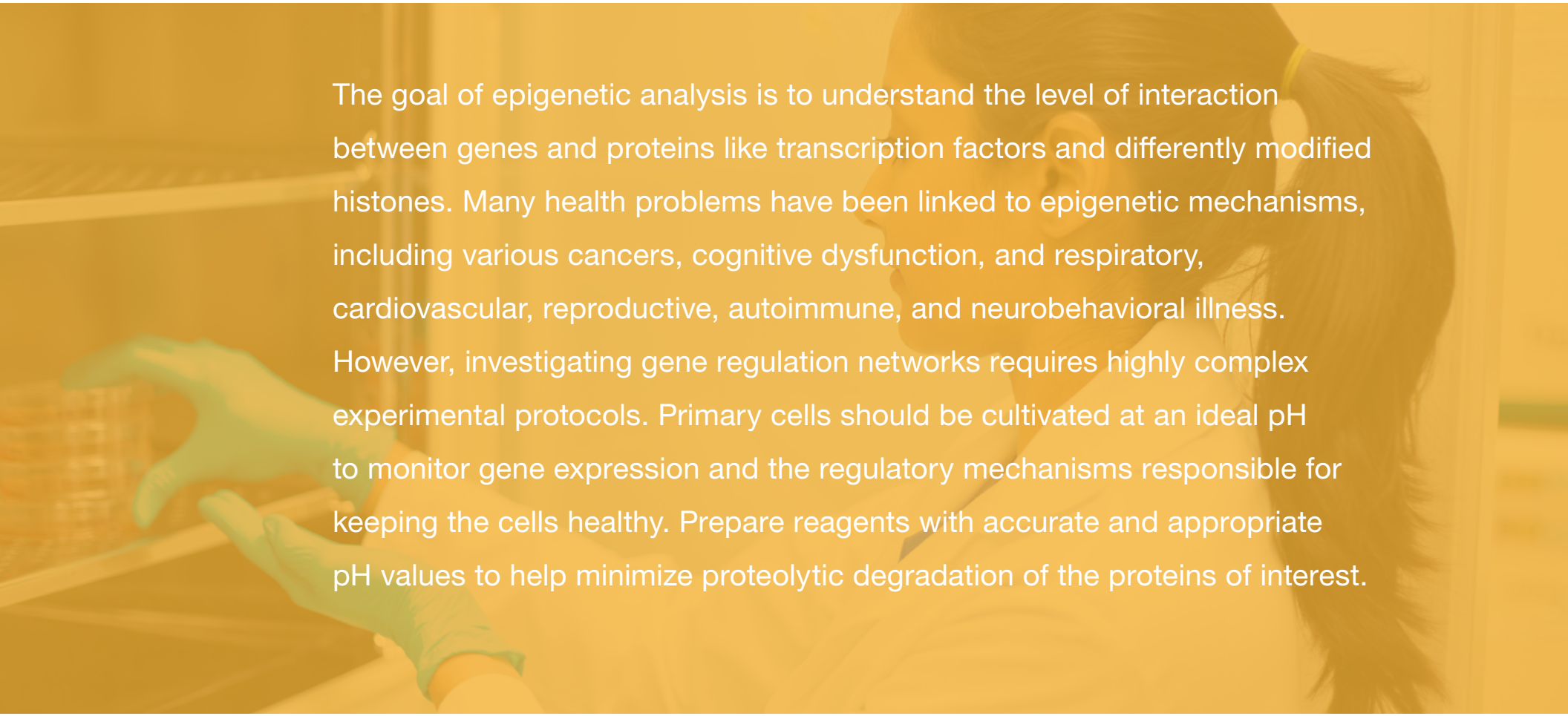


3

Solutions must be at the proper pH when analyzing recombinant proteins.

Use **Orion pH Buffer Individual Use Pouches** to help ensure an accurate buffer reading with each calibration.

4 Precise and accurate pH control is necessary for epigenetic analysis.



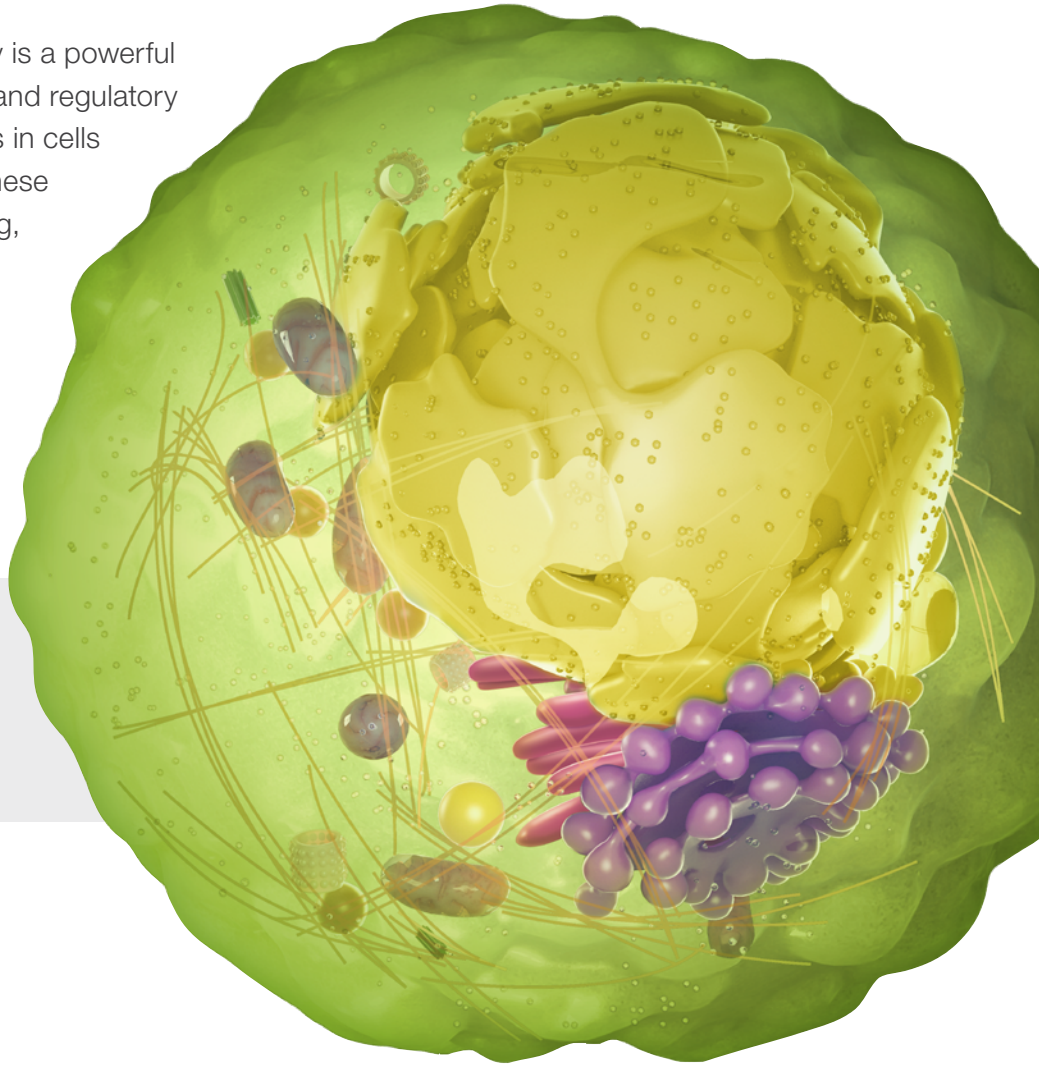
The goal of epigenetic analysis is to understand the level of interaction between genes and proteins like transcription factors and differently modified histones. Many health problems have been linked to epigenetic mechanisms, including various cancers, cognitive dysfunction, and respiratory, cardiovascular, reproductive, autoimmune, and neurobehavioral illness. However, investigating gene regulation networks requires highly complex experimental protocols. Primary cells should be cultivated at an ideal pH to monitor gene expression and the regulatory mechanisms responsible for keeping the cells healthy. Prepare reagents with accurate and appropriate pH values to help minimize proteolytic degradation of the proteins of interest.

4

Precise and accurate pH control is necessary for epigenetic analysis.

The chromatin immunoprecipitation (ChIP) assay is a powerful tool used to capture interactions between DNA and regulatory proteins in their natural states. DNA and proteins in cells are cross-linked with formaldehyde to capture these interactions. To control the extent of cross-linking, the reaction must be efficiently quenched with Tris pH 8.0 buffer. Inconsistent cross-linking is often the cause of variation in ChIP signals. In the later stages of a ChIP assay, the protein of interest is detected with antibodies.

The binding affinities of antibodies can be impacted by the wash buffer composition, salt concentration, and pH.



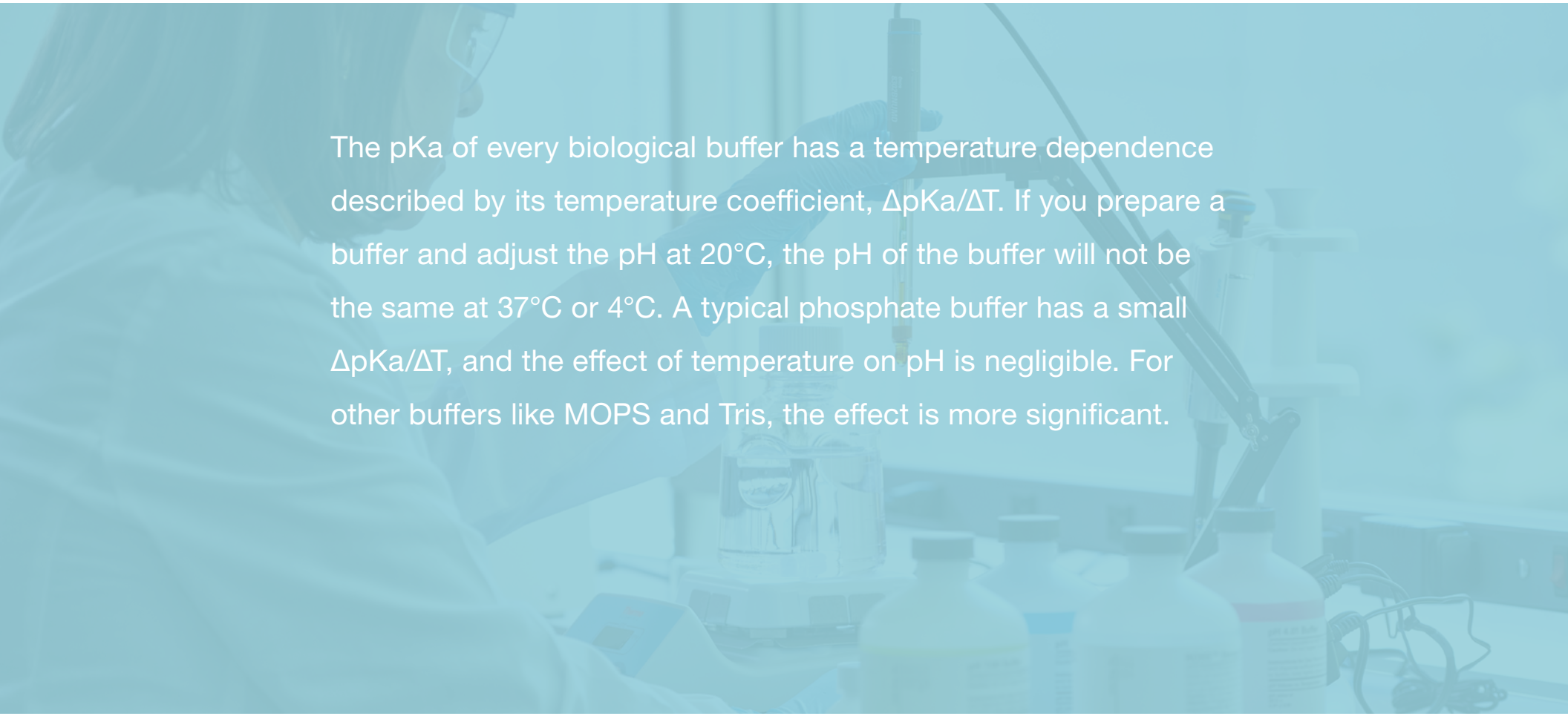
4

Precise and accurate pH control is necessary for epigenetic analysis.

Many other analytical methods are used to detect the footprints of DNA-bound proteins in vitro and in vivo. Sensitivity in the regulatory regions of chromatin to cleavage by dimethyl sulfate or endonucleases can indicate transcription factor occupancy. Oncogenic transcription factors and transcription factors that disrupt tumor suppression pathways are associated with various types of cancer.

Frequent calibration, cleaning, and proper storage of your electrodes in Thermo Scientific™ Orion™ pH electrode cleaning and storage solutions can help ensure your pH measurements remain accurate over time. Soaking your electrodes in Orion pH electrode storage solutions between uses will refresh and restore the glass sensor for fast, accurate readings.

5 The pH of biological buffers can vary significantly with temperature.

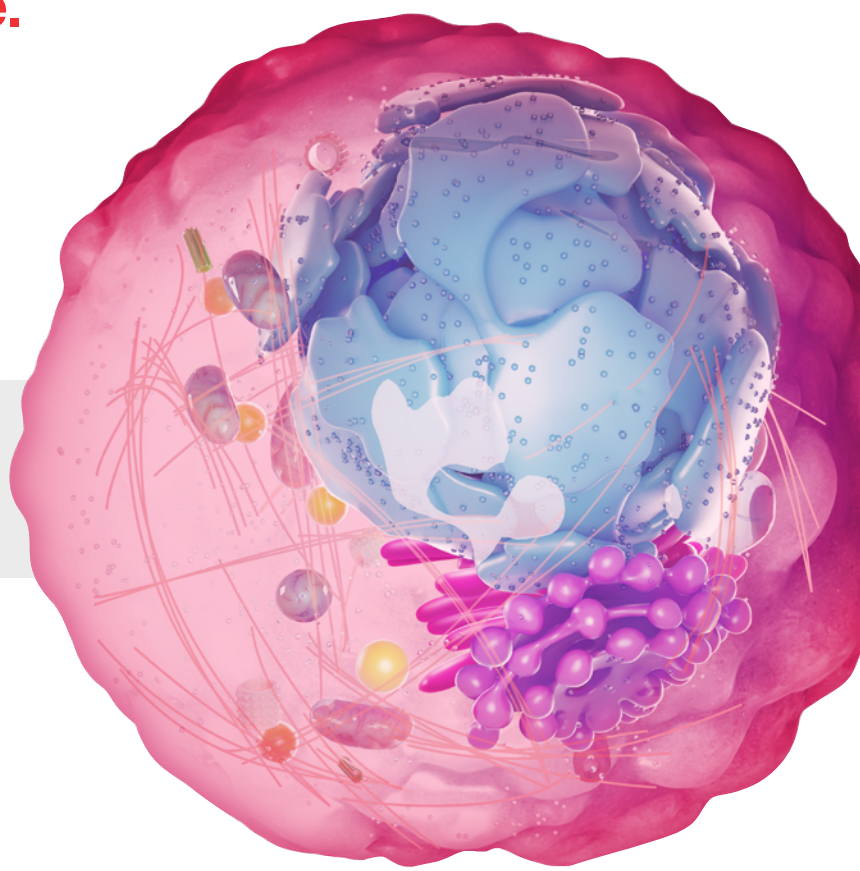


The pKa of every biological buffer has a temperature dependence described by its temperature coefficient, ΔpKa/ΔT. If you prepare a buffer and adjust the pH at 20°C, the pH of the buffer will not be the same at 37°C or 4°C. A typical phosphate buffer has a small ΔpKa/ΔT, and the effect of temperature on pH is negligible. For other buffers like MOPS and Tris, the effect is more significant.

5

The pH of biological buffers can vary significantly with temperature.

Tris buffer has one of the largest pKa temperature coefficients at –0.028 ΔpKa/ΔT. If the pH of Tris buffer is adjusted to 8.30 at 20°C, the pKa will be 8.80 at 4°C and 7.70 at 37°C (Table 1). These are significant shifts that can impact your work.



Failure to take the temperature dependence of biological buffers into account can lead to failed experiments or poor results.

To prevent this from interfering with your work, prepare and adjust the pH of your buffer at the temperature at which you plan to use it. If the work will be performed over a range of temperatures, consider using a buffer with a small pKa temperature coefficient.

Table 1. Dependence of pKa on temperature for some common buffers.

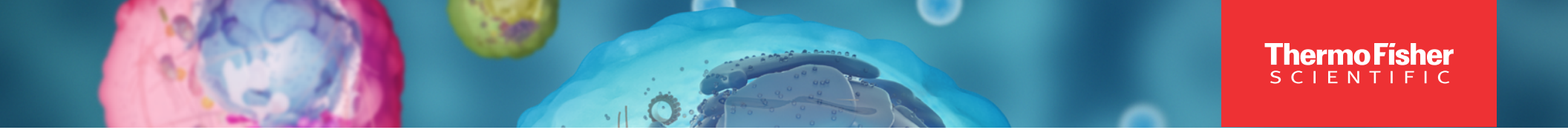
BUFFER	TYPE	ΔpKa/ΔT	pKa AT 4°C	pKa AT 20°C	pKa AT 37°C
MOPS	Biological	–0.011	7.41	7.20	6.98
Tris	Biological	–0.028	8.80	8.30	7.70
Phosphate	Inorganic	–0.0028	7.26	7.21	7.16

5

The pH of biological buffers can vary significantly with temperature.

Using a pH meter like the Thermo Scientific™ Orion™ Versa Star Pro™ Benchtop pH Meter with a Tris-compatible electrode will help ensure that pH measurements always have accurate temperature compensation without the risk of Ag+ or Cl– ions affecting your readings.

An ATC probe or triode will adjust the calibration of the pH electrode to compensate for the change in slope that will occur if the temperature changes. This is essential for accuracy if pH is measured over a range of temperatures. Be aware that pH meter temperature compensation does not adjust for the effect of temperature on the pH of your buffer, because this effect varies from buffer to buffer. Choose an electrode that responds quickly to temperature changes and provides a stable pH reading at your working temperature.



Summary 5 Reasons

Successful cell culture, protein production, and epigenetic analysis all require pH to be optimal each step of the way. Accurate pH measurement and precise control of pH are thus imperative. It is also necessary to monitor the pH of solutions over time and to accurately measure pH at different temperatures to help ensure that pH-dependent processes function properly. Selecting an appropriate pH meter and electrode, knowing how to use them correctly, and taking good care of your equipment are no less important. Following these recommendations will facilitate the growth and maintenance of healthy cells for any application involving cell culture.

Resources

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