

# Boca Scientific's Reagents and Kits Increase Walkaway Time and Speed Up Laboratory Procedures

With the pace of scientific research accelerating at an unprecedented speed, it is vital to eliminate time spent on laborious and routine laboratory procedures and increase walkaway time. Boca Scientific works with iNtRON Biotechnology to provide solutions for several routine laboratory procedures. Our practical laboratory solutions allow scientists in fundamental research and clinical facilities to free up time spent on routine procedures to better spend their time on complex experimental techniques, data analysis and running scientific experiments simultaneously.

# Mycoplasma detection kits

*Mycoplasma* is a genus of bacteria that lack a cell wall and are considered the smallest known cell. In humans, infection from *Mycoplasma pneumonia* can cause severe disease in vulnerable populations, such as hospitalized patients. In a laboratory setting, mycoplasma infection is a widespread problem affecting cell lines.

Mycoplasma-contaminated cells change morphology and grow slower and can also alter gene expression in human cells. Routine detection is needed to ascertain whether mycoplasma is present in a laboratory facility before cells can be treated in a timely manner.

PCR detection of mycoplasma offers several advantages over other traditional methods (such as microbial culture) and rapid tests like biochemical tests. PCR detection is deemed more sensitive, specific and accurate than enzymatic methods and microbial culture. In addition, PCR detection of mycoplasma is also faster than microbial culture and can detect mycoplasma strains that grow poorly or not at all on artificial culture mediums, including *M. hyorhinis* and *M. orale*.

Boca Scientific collaborates with iNtRON Biotechnology to provide a PCR-based mycoplasma detection kit — <u>e-Myco<sup>™</sup> Mycoplasma PCR Detection Kit (ver 2.0)</u>. Both *M. hyorhinis* and *M. orale* <u>strains are detected</u> using the e-Myco Mycoplasma PCR Detection Kit.

For a more detailed analysis of *Mycoplasma* strains, the <u>e-Myco<sup>™</sup> plus Mycoplasma PCR Detection</u> <u>Kit</u> can be used. This kit allows the detection of 8 genera and 209 species of *Mycoplasma*. Following PCR amplification using primers designed from DNA sequences coding for the highly conserved 16 rRNA, the amplified PCR products can be separated and the species defined by sequencing analysis as provided by the sequencing primer sequences. Different species have different sequences. Additional PCR reactions can also be performed using the appropriate primers. The inclusion of three control samples in the e-Myco plus Mycoplasma PCR Detection Kit allows the unambiguous determination of the presence or absence of mycoplasma. The tests are also sensitive, and the e-Myco plus Mycoplasma PCR Detection Kit needs only <u>15 cells or 10 ~ 20 pg of genomic</u> <u>DNA per test</u> to detect mycoplasma.

For this reason, the e-Myco Mycoplasma PCR Detection Kit and e-Myco plus Mycoplasma PCR Detection Kit products from Boca Scientific's partner iNtRON Biotechnology are routinely used in research<sup>1-4</sup>. One recent study published in <u>Cell Reports</u> used the e-Myco plus Mycoplasma PCR Detection to routinely monitor mycoplasma contamination in cell lines. This study described the effect of influenza-MRSA coinfections in the lung microenvironment<sup>5</sup>.

#### Lipopolysaccharide (LPS) extraction kits

Studying LPS from gram-negative bacteria is particularly important for understanding antibiotic resistance, as gram-negative bacteria alter their structure to avoid antimicrobial peptides (AMPs). Boca Scientific provide a solution for the facile extraction of LPS: <u>LPS Extraction Kit (Cat# 17141)</u>, which allows extracts high yields of LPS from a small volume of bacterial cells.

The <u>LPS Extraction Kit (Cat# 17141)</u> can be employed for several research applications, from the systematic study of bacteria and the immune response of carbohydrate antigens to identifying antibiotics.

In a <u>recent study published in PLOS ONE</u>, Boca Scientific's LPS Extraction Kit was essential in studying the effect of a chemical agent, K2<sup>6</sup>. K2 renders the bacteria *K*. *pneumoniae* more susceptible to intracellular killing in phagosomes. The authors showed that K2 achieves this by increasing the permeability of the bacterial cell envelope by perturbing the protective effect of the outer bacterial layer, which includes LPS. However, other elements may be involved.

LPS was extracted from bacteria using the iNtRON Biotechnology kit following the manufacturer's instructions. LPS extracts were separated using gel electrophoresis and visualized by silver staining the separated extracts. The LPS Extraction kit showed that the inactivation of genes perturbing the synthesis of LPS clearly displayed changes in the O-antigen structure of the LPS and was essential in defining the mode of action of K2.

Another <u>recent study published in the Journal of Bacteriology</u> used the LPS Extraction Kit to study the mechanism of action of recipient cells targeted by contact-dependent growth inhibition (CDI) system proteins. CDI occurs when bacterial cells deliver toxins to neighboring bacterial cells upon direct contact, leading to harmful consequences, such as growth arrest or cell death. The LPS Extraction Kit was used in the study to show that recipient cells can only be targeted if they produce full-length LPS<sup>7</sup>.

# Boca Scientific's solutions for DNA and RNA purification

### Automated nucleic acid extraction

Given the constant need for extracting DNA and RNA, automation of the process is important in freeing up turnaround time for clinical and laboratory staff to spend more time on technically demanding experimental processes.

Boca Scientific introduces iNtRON's automated DNA and RNA extraction system to the market, the Miracle-AutoXT system. The <u>Miracle-AutoXT</u> is <u>FDA-registered</u> for fast and efficient nucleic acid extraction. iNtRON's automated system allows the extraction of nucleic acids from various samples and ensures a <u>high purity and extraction rate</u>. The Miracle-AutoXT system prevents crosscontamination, vital for processing pathogenic samples, by using prefilled cartridges or wells for purification.

# Figure 1. Extracting Adenovirus (AdeV) DNA and Norovirus GII (NoV II) RNA from stool samples using the Miracle-AutoXT system and competitors



The Miracle-AutoXT system (AutoXT PGS) offers higher extraction rates than competitor extraction systems.

Boca Scientific also offer an extraction kit suitable for DNA/RNA extraction from pathogens: <u>AutoXT</u> <u>PGS DNA/RNA Extraction Kit</u>. Used together with the automated sample processer, the AutoXT PGS DNA/RNA Extraction Kit employs magnetic bead technology for fast and efficient binding of nucleic acids from various sample types. These include fresh or frozen blood, serum, cell-free fluids, virus cultures, cultivated cells, tissue homogenate and stool swab homogenate.

# **Genomic DNA purification**

Boca Scientific provides several solid-based genomic extraction techniques that are advantageous over solution-based methods as they are less hazardous to the experimental user, such as organic extraction or ethanol precipitation. The <u>G-spin columns</u> are based on advanced silica-gel membrane technology that results in the rapid and efficient purification of DNA from different sources.

| Sample type                        | Boca Scientific's DNA extraction solution   |
|------------------------------------|---|
| Genomic DNA from all sources       | G-spin Total DNA Extraction Kit   |
|                                    | G-DEX IIc Genomic DNA Extraction Kit for Cells and<br>Tissues                                     |
| Genomic DNA from microorganisms or | G-spin Genomic DNA Extraction Kit for Bacteria  |
| cells infected by microorganisms   | i-genomic BYF DNA Extraction Mini Kit   |
|                                    | Patho Gene-spin DNA/RNA Extraction Kit- for Pathogenic<br>samples infected by viruses or bacteria |
|                                    | Viral Gene-spin DNA/RNA Extraction Kit  |
| Genomic DNA from plants            | i-genomic Plant DNA Extraction Mini Kit   |

#### Table 1. Range of genomic DNA extraction kits

# **Purifying plasmid DNA**

Boca Scientific also offer several solutions for **<u>purifying plasmid DNA</u>**, which allow maximum sample recovery with the kits' specialized silica membrane spin-type columns and buffer systems.

- MEGAquick-spin<sup>™</sup> Plus Total Fragment DNA Purification Kit (Cat#: 17290) for DNA extraction from normal or low-melt agarose gels, purification of PCR products or DNA cleanup. DNA is recovered from isolated DNA fragments or PCR products in as little as 20 mins whilst achieving high recovery rates (90-95% for PCR and 70-85% for gel extraction)
- DNA-spin<sup>™</sup> Plasmid DNA Purification Kit (Cat#: 17098) for separating and purifying plasmid DNA from bacteria. The silica membrane spin-type columns bind up to 35 µg of DNA in the presence of high concentrations of chaotropic salt and allow elution with a small volume of low-salt buffer. This eliminates the need for phenol-chloroform extraction and alcohol precipitation.
- <u>Fast DNA-spin<sup>™</sup> Plasmid DNA Purification Kit (Cat#: 17013)</u> for separating and purifying plasmid DNA from *E. coli* in only 10 mins and eliminating the need for ice incubation and centrifugation steps.

# **RNA** extraction

In partnership with iNtRON Biotechnology, Boca Scientific also provide a range of products for RNA extractions. These <u>RNA extraction kits</u> can be used for pathogen detection studies, RNA hybridization protocols and cDNA synthesis.

# Molecular biology solutions

Boca Scientific have several PCR Premixes optimized for specific downstream applications. The PCR Premixes contain all the PCR components, so all that is needed is the template and primers. Additionally, users can directly move from PCR reaction to analysis with gel electrophoresis without any additional steps, as the loading buffer is also included in the tube.

All PCR-premixes (Table 1) are manufactured in a highly controlled environment, and each batch is quality-checked to ensure reproducibility, specificity, and accuracy.

| PCR Premix  | Application  |
|---|--|
| Maxime PCR PreMix Kit (i-Taq)<br>96 Tubes <mark>(Cat# 25025)</mark>                             | <ul> <li>Amplification of genomic DNA, cDNA, to 5 kb</li> <li>Droplet Digital PCR and RT-PCR</li> <li>Human leukocyte antigens (HLA) typing</li> <li>PCR-based DNA typing</li> <li>T/A cloning or blunt-end cloning</li> </ul>   |
| Maxime PCR PreMix Kit (i-<br>StarTaq)<br>96 Tubes <u>(Cat# 25165)</u>                           | <ul> <li>Pathogen detection</li> <li>Genomic DNA PCR</li> <li>Hot-start PCR</li> <li>Real-time PCR</li> <li>High GC-rich, repeat region PCR</li> <li>T/A vector cloning</li> <li>Loss of heterozygosity (LOH)- or Microsatellite Instability<br/>Testing (MSI) analysis-related PCR</li> </ul> |
| Maxime PCR PreMix Kit, pfu<br>(Cat#: 25185)   | <ul> <li>General PCR</li> <li>Template amplification for sequence analysis</li> <li>Blunt cloning</li> </ul>   |
| Maxime PCR PreMix Kit, Long<br>Distance/High Fidelity<br>(Cat#: 25265)                          | <ul> <li>General PCR</li> <li>Human genomic DNA for PCR</li> <li>20kb-long DNA fragment amplification</li> <li>T/A cloning or blunt-end cloning</li> </ul>   |
| Maxime PCR PreMix Kit, Long<br>Distance/High Fidelity with<br>Hot-Start<br><u>(Cat#: 25281)</u> | <ul> <li>General PCR</li> <li>Human genomic DNA for PCR</li> <li>Disease diagnostics</li> <li>Multiplex PCR</li> <li>SNP analysis</li> <li>T/A cloning or blunt-end cloning</li> </ul>   |

 Table 1. PCR Premixes from Boca Scientific

Boca Scientific not only offer premixes for PCR but also for reverse-transcription PCR (RT-PCR).

The Maxime RT PreMix (iNtRON Biotechnology) was recently used in a study published in <u>Nature npj</u> <u>Genomic Medicine</u>, which describes the interplay between tumor microenvironment (TME) and conventional anticancer chemotherapy (gemcitabine). Boca Scientific's RT-PCR premix saved time on RT-PCR protocols, which analyzed the expression levels of *CD24*, *CD44* and *SLC16A1*. The researchers identified that targeting CD44 and the protein product of *SLC16A1*, MCT1, which are associated with enhanced anchorage-independent growth or invasiveness, may be useful in preventing the recurrence of pancreatic cancer in patients treated with gemcitabine<sup>8</sup>.

# Contact us for more information on our diverse range of laboratory reagents and kits

From DNA/RNA purification and LPS extraction to detecting mycoplasma contamination, Boca Scientific have all the laboratory solutions you need. Please <u>contact us</u> if you need help and further information about any of our products.

## Scientific literature employing Boca Scientific's products

- 1. Heller, L. *et al.* Growth environment influences B16.F10 mouse melanoma cell response to gene electrotransfer. *Bioelectrochemistry* **140**, (2021).
- 2. Broadley, K. W. R. *et al.* Side population is not necessary or sufficient for a cancer stem cell phenotype in glioblastoma multiforme. *Stem Cells* **29**, (2011).
- 3. Baulier, E., Garcia Diaz, A., Corneo, B. & Farber, D. B. Generation of a human Ocular Albinism type 1 iPSC line, SEli001-A, with a mutation in GPR143. *Stem Cell Res* **33**, (2018).
- 4. Allman, E. L., Painter, H. J., Samra, J., Carrasquilla, M. & Llinás, M. Metabolomic profiling of the Malaria Box reveals antimalarial target pathways. *Antimicrob Agents Chemother* **60**, (2016).
- 5. Langouët-Astrié, C. *et al.* The influenza-injured lung microenvironment promotes MRSA virulence, contributing to severe secondary bacterial pneumonia. *Cell Rep* **41**, (2022).
- 6. Ifrid, E. *et al.* 5-ethyl-2'-deoxyuridine fragilizes Klebsiella pneumoniae outer wall and facilitates intracellular killing by phagocytic cells. *PLoS One* **17**, e0269093 (2022).
- 7. Elery, Z. K., Oates, A. E., Myers-Morales, T. & Garcia, E. C. Recipient Cell Factors Influence Interbacterial Competition Mediated by Two Distinct Burkholderia dolosa Contact-Dependent Growth Inhibition Systems. *J Bacteriol* **204**, (2022).
- 8. Jang, G. *et al.* Direct cell-to-cell transfer in stressed tumor microenvironment aggravates tumorigenic or metastatic potential in pancreatic cancer. *NPJ Genom Med* **27**, 63 (2022).