

Electroporation optimization

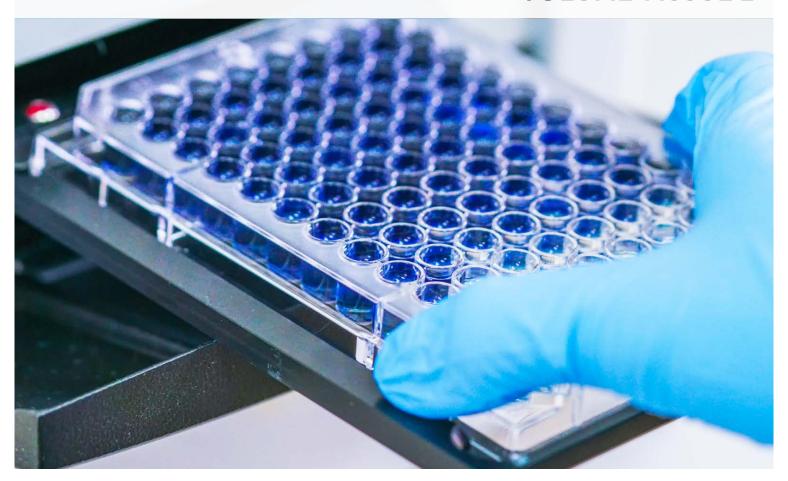
Automated extraction of DNA from various food samples

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**VOLUME 1 ISSUE 2** 



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VWR® Ultra-Low Freezers, 400-Box Capacity	115 V, 60 Hz	400 box (2")	19.4 cu.ft. (Interior)	32.3W×38.4D× 78"H	23.1W×28.3D ×51.2"H	NEMA 5-20	13/20	687 lbs.	76307-948
VWR® Ultra-Low Freezers, 500-Box Capacity	115 V, 60 Hz	500 box (2")	24.1 cu.ft. (Interior)	37.9W×38.4D× 78"H	28.8W×28.3D ×51.2"H	NEMA 5-20	12.5/20	734 lbs.	76307-936
VWR® Ultra-Low Freezers, 600-Box Capacity	115 V, 60 Hz	600 box (2")	28.8 cu.ft. (Interior)	43.5W×38.4D× 78"H	34.4W×28.3D ×51.2"H	NEMA 5-20	12.7/20	820 lbs.	76307-954



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Description	Exterior Dimensions	Temperature Range	Cat. No.
ULT Stirling Undercounter Ultra-	864H x 711D x 686W mm	-86°C to −20°C @ 32°C (90°F) ambient,	
Low Temperature Freezer	(34 x 28 x 27")	adjustable to 1°C increments	75845-818





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115 V, 60 Hz	651 l (23 cu.ft.)	2057H×705W×813D mm (81×27.75×32")	4	178 kg (392 lbs.)	76314-252
115 V, 60 Hz	1387 l (49 cu.ft.)	2057H×1339W×813D mm (81×52.7×32")	8	271 kg (597 lbs.)	76314-260
115 V, 60 Hz	2038 l (72 cu.ft.)	2057H×1924W×813D mm (81×75.7×32")	12	332 kg (733 lbs.)	76314-268





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Description	Capacity	Membrane Diameter	Pore Size	Quantity	Cat. No.
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PVDF Membrane					
Complete Filtration Unit	150 mL	50 mm	0.22 µm	Cs. 12	76010-374
Nylon Membrane					
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Refrigerated Centrifuge, Cell Culture Bundle	8 x 15/50 mL	4500 rpm	3,260 g	4-Place Universal and Adapters	10830-764
Ambient Centrifuges					
Centrifuge, Cell Culture Bundle	8 x 15/50 mL	4500 rpm	3,260 g	4-Place Universal and Adapters	10830-762



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Description	Volume	Cat. No.
CO <sub>2</sub> Incubator Air Jacket TC	6.5 CF	10810-902

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- Clean conditions available two-hour dry H<sub>2</sub>O<sub>2</sub> sterilization cycle, tool-free three-minute interior removal, opt. controlled humidification system
- Facility-friendly easy-use large color touchscreen, standard power plugs & low power usage, 25 cu.ft. units roll through doorways.

Volume, L (cu. ft.)	Electrical	Cat. No.
708 (25)	115V, 60Hz, 16A	10158-720
708 (25)	208-230V, 60Hz, 10A	10158-842
934 (33)	115V, 60Hz, 16A	10158-846
934 (33)	208-230V, 60Hz, 10A	10158-734





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VWR® Multistage Gas Regulators feature safe, accurate, and sensitive delivery of reduced pressure.

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Carbo	on Dioxide	Carbon Dioxide	_	1-50 psi	_	320	55850-414



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Description	Size (Or Another Secondary Description)	Unit (Each, Pack, or Case)	Cat. No.
Portable Pipet-Aid XP Controller		Each	53498-105
Portable Pipet-Aid with Gravity Drain		Each	53440-188
Portable Pipet-Aid XP2		Each	89166-464
Portable Pipet-Aid XL		Each	53439-990

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Description	Cat. No.	Free 2nd Purification Pk.
	10034-540, 10034-542, 10034-544, 10034-546, 10034-548, 76048-804, 76048-806,	10035-894, 10035-890,
PURELAB Chorus	76048-808, 76048-810, 76048-796, 76048-798	76048-854, 76048-852
PURELAB Classic	89204-072, 89204-076, 89204-080, 89204-084	89204-376
PURELAB Flex	89204-088, 89204-092, 89221-838, 89221-840, 89221-842, 89221-844	89204-392, 89204-404



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Description	Electrical	Sash Opening	Cat. No.
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- Ultra harmonic technology
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- Sample protection
- Time efficiency
- Multi-dimension imbalance tolerance

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Description	Electrical	Includes	Cat. No.
Avanti J-15R, Refrigerated, Benchtop		Includes benchtop centrifuge and BioCertified JS-4.750 (4 $\times$ 750 mL) swinging bucket rotor. (Rotor is BioCertified when used with bucket	
Centrifuge Swinging		covers or aerosolve canisters.) Adapters, tubes/bottles, bucket covers and	
Bucket Package	120 V, 60 Hz	aerosolve canisters are NOT included and can be ordered separately.	BKC19397
Avanti J-15R,		Includes benchtop centrifuge and BioCertified S-4.750 (4 x 750 mL) swinging bucket rotor, BioCertified aerosolve canisters (qty 4), 15 mL conical BioSafe	
Refrigerated,		aerosolve canister tube racks (qty 4) and 50 mL conical BioSafe aerosolve	
Benchtop Centrifuge		canister tube racks (qty 4). (Rotor is BioCertified when used with aerosolve	
BioSafe Package	120 V, 60 Hz	canisters.) Tubes/bottles are NOT included and can be ordered separately.	BKC19409



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#### THE FIRST AUTOMATED CELL COUNTER THAT UTILIZES ONLINE IMAGE PROCESSING WITH A CLOUD-BASED MACHINE LEARNING ALGORITHM



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Description	Cat. No.
Corning® Cell Counter	76200-994



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FASTTRACK™ TECHNOLOGY ALLOWS FOR SPEEDY MEASUREMENTS OF METILER THE ENTIRE SPECTRUM ALL WITHIN A COMPACT FOOTPRINT

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UV5 Excellence UV/VIS Spectrophotometer	20.8W x 25.5D x 25.4H cm (8.19 x 10.04 x 9.98")	>1.5 (Toluene in Hexane)	10811-200
UV5Bio Excellence UV/VIS Spectrophotometer	20.8W x 25.5D x 25.4H cm (8.19 x 10.04 x 9.98")	>1.5 (Toluene in Hexane)	10811-202
UV5Nano Excellence UV/VIS Spectrophotometer	20.8W x 25.5D x 21.7H cm (8.19 x 10.04 x 8.54")	>1.7 (Toluene in Hexane)	10811-204



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Our cryogenic storage tubes and accessories are designed as a system to protect and organize your valuable, irreplaceable samples.

Thermo Scientific Nalgene® Externally Threaded and Thermo Scientific Nunc® Internally Threaded Cryogenic Vials

		Size (Or Another	
Description	Unit (Each, Pack, or Case)	Secondary Description)	Cat. No.
1.2 mL Nalgene® Cryogenic Vials, External			
Thread with Screw Cap	Case of 500	25/Bag	66008-706
1.2 mL Nalgene® Cryogenic Vials, External			
Thread with Screw Cap	Case of 1,000	Bulk	66008-751
2 mL Nalgene® Cryogenic Vials, External Thread			
with Screw Cap	Case of 500	25/Bag	66008-728
2 mL Nalgene® Cryogenic Vials, External Thread			
with Screw Cap	Case of 1,000	Bulk	66008-754
5 mL Nalgene® Cryogenic Vials, External Thread			
with Screw Cap	Case of 250/Pack of 10	10/Bag	66008-732
1 mL Nunc® Skirted Self-Standing Vials, Conical			
Bottom, Internally Threaded	Case of 2000	500/Pack	66021-992
1.8mL Nunc® Skirted Self-Standing Vials, Round			
Bottom, Internally Threaded	Case of 1800	450/Pack	66021-987
1 mL Nunc® Starfoot Self-Standing Vials, Conical			
Bottom, Internally Threaded	Case of 2000	500/Pack	66021-993
1.8mL Nunc® Starfoot Self-Standing Vials Round			
Bottom, Internally Threaded	Case of 1800	450/Pack	66021-986
3.6mL Nunc® Starfoot Self-Standing Vials,Round			
Bottom, Internally Threaded	Case of 1600	400/Pack	66021-989
4.5mL Nunc® Starfoot Self-Standing Vials, Round			
Bottom, Internally Threaded	Case of 1200	300/Pack	66021-991





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- Capture software will deliver high quality quantifiable data

Up to four solid state lasers (488, 520, 658 and 784 nm) offering ultimate excitation sensitivity. Photon multiplier tube (PMT) for fluorescence and phosphor imaging, avalanche photodiodies (APD) for near-infrared imaging and a CCD sensor for chemiluminescent and visible imaging. Ultra-wide dynamic range for imaging and quantifying low and high abundance samples simultaneously. Image resolution down to 10 microns for high-quality image analysis.

Description	Cat. No.
Sapphire Biomolecular Imager - NIR	76317-604



#### INGENIO® EZPORATOR® ELECTROPORATION SYSTEM, MIRUS BIO





- Performance: deliver any nucleic acid to hard-to-transfect, stem and primary cells
- Simplicity: use a single, universal electroporation solution across all cell types
- Flexibility: easily optimize electroporation parameters for each cell type

Electroporation is the method of choice for many hard-to-transfect cell types, and the Ingenio® EZporator® Electroporation System is a cost-effective, straightforward, open system that is perfect for any lab seeking performance without breaking the bank.

Description	Cat. No.
Ingenio® EZporator® Electroporation System	76304-532

This specific product is not available in Canada. Please contact your VWR Sales Representative to learn about easy access to similar options available in your region.

# Electroporation optimization:

### For more information, visit vwr.com/ mirusbio

# A user's guide

By Anthony Lauer and Laura Juckem, Mirus Bio LLC, Madison, Wisconsin USA

#### **ABSTRACT**

Electroporation is an effective physical method for delivery of various types and sizes of molecules to cells in culture. It is a fast and relatively easy way to re-engineer cells to contain exogeneous nucleic acids or small molecules. Electroporation conditions, including strength of the pulse and buffer composition, can have profound effects on the delivery efficiency and cellular viability post-electroporation, thereby influencing the total number of cells available for experimentation.

#### INTRODUCTION

Electroporation is the application of an electric field pulse that result in changes in the cellular membrane, promoting the uptake of exogenous macromolecules including DNA, RNA and proteins. Plasmid DNA is commonly electroporated into mammalian cells to overexpress a gene of interest. Cell and molecular biologists commonly utilize electroporation methods due to its low cost, ease-of-use and high efficiency delivery of various types of cargo. The mechanism of electroporation of mammalian cells is poorly understood; however, the most recognized theory suggests that the rapid administration of an electrical pulse creates transient leaky structures or pores in the cellular membrane that allow entry of the nucleic acid or other macromolecules into the cell interior. The DNA, RNA or protein do not require modification or complexing with lipids or polymers which can be beneficial for therapeutic applications. The key experimental factors for mammalian electroporation experiments include: nucleic acid and cell concentration, pulse strength, and buffer composition. Each cell type has specific experimental conditions that will allow for high efficiency electroporation of the macromolecule of interest with minimal cytotoxicity.

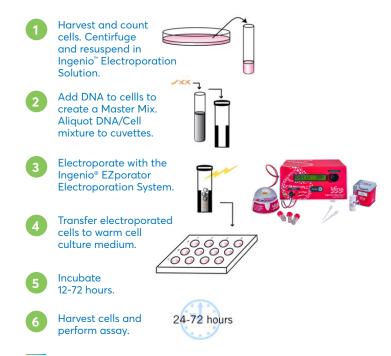


FIGURE 1: Overview of electroporation workflow

Electroporation devices can solicit three types of electrical wave forms including: exponential decay, square wave and time constant. Exponential decay pulses can be optimized by varying the voltage and capacitance settings to achieve a wide pulse gradient. Square wave pulses are characterized by the voltage, time of the pulse, duration between pulses, and the total number of pulses. Time constant pulses specify a single sustained voltage for a designated time. Exponential and square wave are the most common pulses utilized for mammalian electroporation studies.



Specificly, an exponential decay pulse releases the charge from a capacitor set to a specific maximum voltage (Vmax), which then decays exponentially, typically over milliseconds. The delivered pulse is characterized by the field strength (kV/cm) and the time constant (T) which is the time elapsed before the voltage reaches 1/3 Vmax. The time constant is influenced by both the set capacitance and resistance of the system.

#### MAMMALIAN CELL ELECTROPORATION PROTOCOL

The process of electroporating mammalian cells is straightforward (Figure 1). For all conventional electroporators, the cells are harvested from the tissue culture plate, counted and resuspended at a high density in the electroporation buffer. The recommended cell density depends on the size of the cells, 1 x 10<sup>7</sup> cells per ml for smaller cells such as immune cells, 5 x 10<sup>6</sup> cells/ml for larger cells such as Hela. The plasmid DNA, or other macromolecule to be delivered to the cells, is added to the cell mixture and transferred to the electroporation cuvette. This contains the electrodes that will conduct the pulse generated from the electroporation device. The cells are then electroporated at a designated voltage and capacitance. Post-electroporation the cells are transferred to a tissue culture plate containing warm complete growth medium. The incubation and harvest time will vary depending on the nature of the experiment. Typically, cells are harvested 24-72 hours post-electroporation and assayed for gene expression or gene regulation.

#### **Pulse Strength**

A key parameter to achieve successful permeabilization of the cell membrane is the Field Strength (E) that is applied to the cell. Field strength is calculated using the initial peak voltage that is applied during an exponential decay pulse divided by the gap size of the cuvette (kV/cm). Mammalian cells are less tolerant of high voltage settings than prokaryotes, so a lower field strength must be applied to maintain cell viability. Typically starting voltages range from 80-160 V for a 0.2 cm cuvette or 200 – 300 V for a 0.4 cm cuvette.

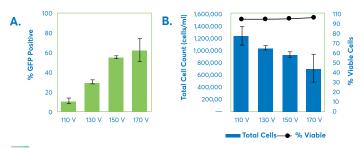


FIGURE 2: Pulse strength affects electroporation efficiency and total cell count. Jurkat E6-1 cells were electroporated with an EGFP reporter plasmid at varying voltages using the Ingenio® Electroporation Solution (Mirus Bio) and the Ingenio® EZporator® Electroporation System (Mirus Bio). GFP efficiency (A) or cell count and viability (B) using propidium iodide was determined by flow cytometry at 48 hours post-electroporation.

Titration of the pulse strength was performed in Jurkat E6-1 cells using 0.2 cm cuvettes and the Ingenio® Electroporation Solution with the Ingenio® EZporator Electroporation System (Figure 2A). Three parameters were assessed including: (1) the effectiveness of the gene delivery (% GFP positive cells), (2) number of viable cells, and (3) total cell count. The goal in a voltage titration experiment is to determine the voltage conditions that maximizes these three parameters to achieve high transgene expression and many healthy cells.



FIGURE 3: Buffer composition influences electroporation efficiency and cell viability. Cells were electroporated in parallel with an EGFP reporter plasmid using either (A) Ingenio® Electroporation Solution or Phosphate Buffered Saline (PBS) on the Gene Pulser Xcell<sup>™</sup> Eukaryotic System (Biorad) or (B) Ingenio® Electroporation Solution or competitor electroporation kit using Amaxa® Nucleofector® II Device (Lonza). GFP efficiency and cell viability using propidium iodide was determined by flow cytometry at 24 hours post-electroporation.

#### **Electroporation Buffer**

The composition of the buffer utilized during the electroporation process is critical to achieving high efficiency and low toxicity results. Phosphate buffer saline (PBS) or cell culture medium is frequently used as an electroporation buffer; however, these can have detrimental effects on plasmid DNA delivery efficiency and cell viability (Figure 3A). We observed a lower number of GFP positive cells, and lower cell viability measured by propidium iodide exclusion, when Jurkat E6-1, K562 or HL-60 cells were electroporated in a PBS solution compared to the Ingenio® Electroporation Solution (Mirus). Another commonly used solution is the Nucleofector® Solution V (Lonza) in combination with the Amaxa® Nucleofector IIb Device. Similar plasmid DNA efficiencies and cell viabilities were observed between the Ingenio® Electroporation Solution or the Nucleofector® Solution V in Jurkat E6-1, K562 or HL-60 cell types when electroporated using the same program on the Amaxa® Nucleofector® IIb Device (Figure 3B).



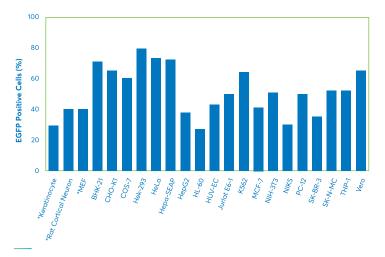


FIGURE 4: Efficient plasmid DNA delivery to a wide variety of cell types. Ingenio® Electroporation Kits were used to transfect indicated cell types using the Amaxa® Nucleofector® IIb Device (Lonza). Cells were assayed at 24 hours by flow cytometry and bars represent the percentage of live cell population expressing GFP. The cell types with an asterisk are primary cells.

#### **Cell Type Specificity**

The maximum transfection efficiency that can be obtained for different cell types is based on factors including their size and membrane composition, so field strength must be selected for each cell accordingly. Optimization of electroporation conditions involves the manipulation of pulse parameters such as: voltage, capacitance and resistance. Application of the optimal field strength allows for the most effective electropermeabilization of the membrane, and thereby delivery of nucleic acid and/or protein. Figure 4 demonstrates representative electroporation efficiencies using optimized pulses to deliver plasmid DNA in twenty-two common cell types including primary keratinocytes, rat cortical neurons and mouse embryonic fibroblasts (MEF).

#### **DELIVERY OF DIFFERENT MACROMOLECULES**

While plasmid DNA is the most common macromolecule, the same electroporation conditions can also be utilized to deliver macromolecules of other sizes.

#### **siRNA**

RNA interference (RNAi) is mediated by small (21-23 base pair) double stranded RNA molecules that interact with cellular proteins and mRNA in the cytoplasm. A luciferase system can be utilized to determine the quantitative levels of RNAi knockdown of an exogenous gene.

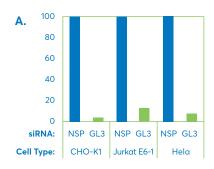
Three cell lines (CHO-K1, Jurkat E6-1 or Hela) were coelectroporated with plasmid DNA encoding GL3 luciferase and either a non-targeting siRNA control or a GL3 targeting siRNA using the Ingenio® Electroporation Solution. Luciferase expression was measured 24 hours post-electroporation and knockdown was

calculated by normalizing expression levels to the non-targeting control (Figure 5A). Greater than 80% knockdown of luciferase was observed in all three cell lines.

#### CRISPR-Cas9

Genome editing using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) involves the delivery of an endonuclease, Cas9, and a gRNA specific to the genomic DNA site for cleavage. Electroporation can effectively deliver Cas9 and gRNA in various forms, including ribonucleoprotein (RNP) complex comprised of an intact Cas9 protein and a synthetic gRNA.

Electroporation of an RNP complex was performed to target the WT1 Associated Protein (WTAP) locus in K562 or Jurkat E6-1 cells using the Ingenio® Electroporation Solution (Figure 6B). Triplicate 0.2cm cuvettes demonstrate high cleavage at the WTAP locus in both immune cell lines; CRISPR cleavage efficiencies range from 28-58%.



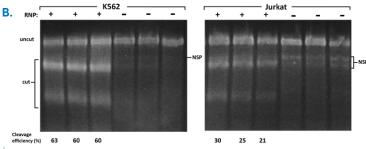


FIGURE 5: Electroporation Can Effectively Deliver siRNA and CRISPR RNP Complexes (A) siRNA and plasmid DNA were co-electroporated with the indicated cell lines with the Ingenio electroporation solution in 0.2 cm cuvettes using either the Gene Pulser Xcell™ Eukaryotic System (Bio-Rad), Plasmid encoding Firefly Juciferase (10ua/ml) was co-electroporated with 250nM of either non-targeting control siRNA or GL3 siRNA into Jurkat E6-1, HeLa or CHO-K1 cells. Twenty-four hours post electroporation, cells were harvested and assayed for luciferase activity. Data was normalized to the non-targeting control siRNA. (B) CRISPR ribonucleoprotein (RNP) complexes targeting WTAP, composed of 750 nM Cas9 protein (NEB) and 1500 nM pre-complexed two-part gRNA (IDT), was electroporated into K562 and Jurkat E6-1 cells using the Ingenio® Electroporation Solution (Mirus Bio) and a Gene Pulser Xcell™ Eukaryotic System (Bio-Rad). Exponential pulse conditions of 150V and 950  $\mu F$  for K562 or Jurkat E6-1 cells were applied to triplicate 0.2 cm cuvettes, 100  $\mu$ l volume, 10 x 106 cells/ ml +/- RNP complex. A T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection. Densitometry was used to quantitate the cleavage efficiency

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120V, 1000W, 8.3A	25.1 x 37.6 x 10.9	17.8 x 17.8	2500	Aluminum	97042-646
120V, 1000W, 8.3A	25.1 x 37.6 x 10.9	17.8 x 17.8	2500	Ceramic	97042-642
120V, 1550W, 12.9A	33 x 45.5 x 10.9	25.4 x 25.4	6000	Aluminum	97042-686
120V, 1550W, 12.9A	33 x 45.5 x 10.9	25.4 x 25.4	6000	Ceramic	97042-682

Length (cm)	Cat. No.
45.7	11301-110

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- AzureSpectra ms700/rb800 Western Kit
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- Goat-anti-mouse HRP secondary antibody
- Low fluorescence western membrane (PVDF) 9x7cm & 10x15cm
- Nitrocellulose transfer membrane, 0.22µm & 0.45µm
- Opaque incubation tray, 9x6cm





Model	Wavelength, nm	Cat. No.
c400	302, 365, 460, 470, 526, 628	10147-218
c500	302, 365, 460, 660, 785	10147-216
c600	302, 365, 460, 470, 526, 628, 660, 785	10147-214
	c400 c500	c500 302, 365, 460, 660, 785

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PeproGrow EPC, Endothelial Progenitor Cell Media	1 Kit	MP700-EPC
PeproGrow MacroV, Macrovascular Endothelial Cell Media	1 Kit	MP700-MACROV
PeproGrow MicroV, Microvascular Endothelial Cell Media	1 Kit	MP700-MICROV
PeproGrow HEK293 Media	1L	MPAFCDHEK293
PeproGrow AF-CHO Media	1L	MPAFCHO
PeproGrow-1 Serum-Free Cell Culture Supplement Kit	1 Kit	MP700-C100
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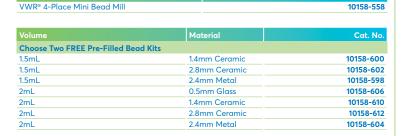
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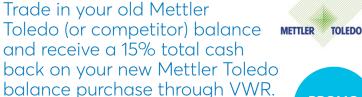
The most powerful bead mill homogenizer available. Supports simultaneous processing of samples in 12 x 0.5 mL, 4 x 1.5 mL, 12 x 2 mL, or 4 x 7 mL samples.

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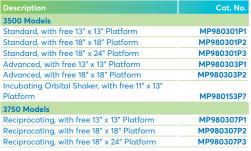
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50001 Incubating, with free 18" x 18" Platform	MP980330P2
5000IR Incubating/Refrigerating, with free 18" x 18" Platform	MP980332P2
10000 Models	
10000-1 Advanced, with free 24" x 24" Platform	MP980320P4
10000-2 Advanced, with free 24" x 24" Platform	MP980322P4

Cat. No.
MP980324P6
MP980326P6
MP980303CO2P1
MP980303CO2P2
MP980303CO2P3







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Mix-n-Stain CF450 Antibody Labelina Kit	20 - 50 ug	405/460 nm	75832-636



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<b>Description</b>	Cat. No.
CytoFLEX System B4-RO-VO	76183-344





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Description	Description	Cat. No.
Rebel Microscope Body	Rebel Microscope Body	76307-540



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Fluorescent Microscope	REVOLVE4 Upright, Inverted, Brightfield, Fluorescent Microscope	76299-574





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Dispensette S analog w/Recirculation Valve	1-10mL	each	10018-952
Dispensette S analog w/Recirculation Valve	2.5-25mL	each	10018-954
Dispensette S analog w/Recirculation Valve	5-50mL	each	10018-956
Dispensette S analog w/Recirculation Valve	10-100mL	each	10018-958
Dispensette S analog w/o Recirculation Valve	0.1-1mL	each	10018-960
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Dispensette S analog w/o Recirculation Valve	0.5-5mL	each	10019-012
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Dispensette S analog w/o Recirculation Valve	2.5-25mL	each	10019-016
Dispensette S analog w/o Recirculation Valve	5-50mL	each	10018-962
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Description	Cat. No
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Captair 391 Smart	75784-718
Captair 481 Smart	75784-720
Captair 392 Smart	75784-724
Captair 483 Smart	75784-726
Captair 633 Smart	75784-634
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230 VAC Unit		
VWR® Power Supply	10 - 500 V	76196-456



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Electrophoresis Blotting System with Gel Wrap					
Handcasting Bundle, 0.75 mm x 10 Well	5.23 mm	0.75 mm	0.75 mm	10	95045-130
Electrophoresis Blotting System with Multiple Gel					
Casting Chamber Bundle, 0.75 mm x 10 Well	5.23 mm	0.75 mm	0.75 mm	10	95045-142

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TotalBLOT+™ PVDF MEMBRANES	10 x 10 cm2	0.2 μm	97062-898
TotalBLOT+™ PVDF MEMBRANES	1 Roll (30 cm x 3 m)	0.2 µm	97062-900
TotalBLOT+™ PVDF MEMBRANES	5 x 15 cm2	0.2 μm	97062-902



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Description	Capacity	Speed	Cat. No.
VWR® Mini Tube Rocker, Fixed Speed	8 Tubes, 120 mm in length	18 rpm	10159-756
VWR® Mini Tube Rocker, Variable Speed	8 Tubes, 120 mm in length	5-25 rpm	10159-752
VWR® Tube Rocker, XL, Variable Speed	18 Tubes, 120 mm in length	5-25 rpm	10159-754



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Description	Electrical	Darkroom Configuration	Camera	Cat. No.
115 V Units				
UVP ChemStudio PLUS 615	115 V	Slide2Hide Door	3.2 MP 615 Camera, Automated 25 mm f/0.95 lens	76307-478
230 V Units				
UVP ChemStudio PLUS 615	230 V	Slide2Hide Door	3.2 MP 615 Camera, Automated 25 mm f/0.95 lens	76307-480

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Description	Cat. No.
PCR Workstation	10783-132



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Description	Size	Cat. No.
Kits		
Monarch Plasmid Miniprep Kit	50 Preps	102971-698
Monarch Plasmid Miniprep Kit	250 Preps	102971-696

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Description	Format	No. of Preparations	Sample Size	Elution Volume	Cat. No.
Direct-zol™ RNA Miniprep Kit	Spin Column	50 Preps	<5×106 Cells	>25 ul	76020-642

This specific product is not available in Canada. Please contact your VWR Sales Representative to learn about easy access to similar options available in your region.



#### **Q QPCR INSTRUMENT, QUANTABIO**

Quantabio

#### A FASTER, SMALLER, BETTER WAY TO QPCR

- Ultra-fast data acquisition 35 cycles in 25 minutes
- Unrivaled performance detect two fold expression level differences
- Portable & compact 4.5 lbs transport without ever calibrating
- Scalable & wireless connect up to 10 instruments (48 samples/instrument)

Q uses a patented magnetic induction technology to rapidly heat samples coupled with fan forced air for cooling to acquire data in only 25 minutes.

Description	Size	Cat. No.
Q 2-channel qPCR Instrument	1 instrument	76175-392
Q 4-channel qPCR Instrument	1 instrument	76175-394



#### ARIAMX REALTIME PCR SYSTEM, AGILENT TECHNOLOGIES



#### FULLY INTEGRATED QUANTITATIVE PCR AMPLIFICATION, **DETECTION, AND DATA ANALYSIS SYSTEM**

- Gene expression analysis
- New genotyping/HRM capability
- mRNA quantification
- NGS quantification: library preparation, result validation

The system design combines a state-of-the-art thermal cycler, an advanced optical system with an LED excitation source, and complete data analysis software.

Description	Details	Cat. No.
Base Instrument		
	Base instrument does not include any optical cartridges. Purchase of	
AriaMx Real-Time PCR System	at least one optical cartridge is required for a functional instrument.	76193-704



#### QTOWER3 REAL-TIME PCR (QPCR) SYSTEMS, ANALYTIK JENA



#### AN OPEN PLATFORM QPCR SYSTEM, BOTH WITH REGARD TO PLASTICWARE FORMATS AS WELL AS KITS/MASTERMIXES

- System is operable via built-in touch screen or external computer
- Corrosion resistance and high temperature homogeneity
- Absolutely no periodic calibration or maintenance for the life of the system

The qTOWER3 Real-Time PCR system from Analytik Jena is an open platform qPCR system, both with regard to plasticware formats (standard 96 well SBS plates, 0.2 ml tubes or 8-well strips) as well as kits/mastermixes

Description	Cat. No.
qTOWER <sup>3</sup> Real-Time PCR (qPCR) System	10861-540

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# Automated extraction of DNA from various food samples

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#### INTRODUCTION

Quality food and its impact on health is becoming more important to costumers. This aspect together with food scandals of the recent past push the food industry to make their products more transparent. One important issue is the origin of raw materials. This point touches topics like the use of materials derived from genetically modified organisms as well as the use of the correct materials indicated on the list of ingredients. An effective method to investigate the origin of materials and genetic background of ingredients in processed food are technologies based on Polymerase Chain Reaction (PCR), as these methods are very sensitive. In order to use PCR-based technologies for the analysis of food, a certain amount and quality of DNA is required. Some sample materials (e.g. plant samples, processed food) contain significant levels of inhibitors, which inhibit PCR-based downstream applications and therefore interfere with analysis.

In the experiments described below, DNA was extracted from northern shrimp, hazelnut and two different kinds of cheese. DNA was extracted using innuPREP DNA kit-IPC16 and innuPREP food DNA kit-IPC16. Extraction was performed with InnuPure® C16/C16 touch. After homogenization (optional) and lysis, lysed samples were transferred in the reagent plastics of the kits. Reagent plastics of the kits are pre-filled with all required buffers and magnetic particles for DNA extraction.

The reagent plastics were loaded in the InnuPure® C16/ C16 touch followed by fully automated extraction. Following extraction, DNA quality was analyzed using gel electrophoresis, spectrophotometry and quantitative real-time PCR (qRT-PCR) for species analysis.

The high pre-processing state of the kits (pre-filled, sealed buffer reservoirs) and automated purification reduces the hands-on time of the customer to a minimum. Besides the benefit of reduced time consumption, pre-processing also reduces the risk of human errors and loss of valuable samples. Analysis of extracted DNA by agarose gel electrophoresis, spectrophotometry and qRT-PCR analysis shows that DNA extracted with devices and kits from Analytik Jena can be readily used for relevant downstream applications.



#### **MATERIALS AND METHODS**

Frozen northern shrimps (50 mg) were homogenized in 200  $\mu$ l H<sub>2</sub>O for 15 seconds using SpeedMill PLUS and Lysis tubes P (Analytik Jena). Homogenates were mixed with 200 µl lysis Solution CBV and 20 µl Proteinase K and incubated at 50°C shaking with 600 rpm for 60 min. 400 µl of the lysate were transferred to the reagent plastic of the innuPREP DNA kit- IPC16 followed by extraction with InnuPure® C16.

100 mg and 200 mg ground hazelnut was resuspended in 800 µl lysis solution CBV and 20 µl Proteinase K. The samples were lysed for 60 min at 65°C. After lysis, 400 µl supernatant were used for extraction with the innuPREP food DNA kit-IPC16 and InnuPure® C16.

DNA was extracted from two varieties of commercially available cheese. Cheeses were declared to be derived from goat and cow milk, respectively. Extraction was performed in quadruplicates using InnuPure® C16 touch and innuPREP food DNA kit- IPC16. 200 mg of cheese were used for each replicate. Elution volume was set to 100 µl. Following extraction, extracts were analyzed by photometry using ScanDrop® 250 and gRT-PCR. For gRT-PCR qTOWER3 and innuDETECT cheese assay were used. The analysis was performed with endpoint method provided by gPCRsoft.

#### **Samples and Reagents**

- innuPREP DNA kit-IPC16
- innuPREP food DNA kit-IPC16
- Lysis tubes P
- innuDETECT cheese assay
- Commercially available kit for hazel analysis

#### Instrumentation

- InnuPure® C16
- InnuPure® C16 touch
- SpeedMill PLUS
- BioShake iQ
- ScanDrop® 250
- Standard equipment for agarose gel electrophoresis and gel documentation
- qTOWER3 G

freezing. Besides degraded DNA, quite some high molecular weight DNA is visible as a distinct band with a size larger than 1500 bp.

After DNA extraction from 100 mg and 200 mg ground hazelnut with the innuPREP food DNA kit-IPC16, extracts were used for gRT-PCR-based detection of hazelnut DNA. In order to detect inhibitory effects of substances contained in the extracted DNA, aliquots of the extract were diluted 1:10 followed by amplification of 1 µl undiluted and 1:10 diluted extract.

Figures 2 and 3 show that DNA extraction from both, 100 mg and 200 mg ground hazelnut, is possible and yields DNA which can readily be used for qRT-PCR. Sample materials with high contents

#### **RESULTS AND DISCUSSION**

After extraction of DNA from frozen shrimp samples, DNA quality and integrity was assessed by spectrophotometrical analysis with ScanDrop® 250 and agarose gel electrophoresis.

Sample number	A260/A280	c [ng/µl]	cmean±SD [ng/µl]
1	2.32	23.64	
2	1.96	21.26	22.1±1.8
3	2.25	23.51	22.111.0
4	1.87	19.94	

Table 1: Spectrophotometrical analysis of DNA extracted from shrimp samples.

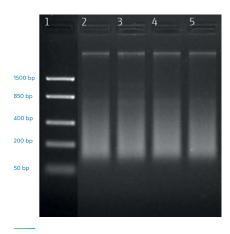


FIGURE 1: Agarose gel electrophoresis of DNA extracted from frozen shrimp samples. Lane 1: DNA ladder; lanes 2–5: Samples 1–4.

The innuPREP DNA kit-IPC16 in combination with the InnuPure® C16 enables the automated DNA extraction from shrimps with minimal hands-on time. As a result of extraction, pure DNA with A260/A280 ratios ranging from 1.87 to 2.32 is obtained. Mean yields of 22.1 ng/ul are sufficient for PCR-based downstream applications. Agarose gel analysis shows that a part of the DNA is degraded which appears as a smear

in lanes 2–5 of Figure 1. DNA degradation is a natural process which starts shortly after the death of the animals and stops with

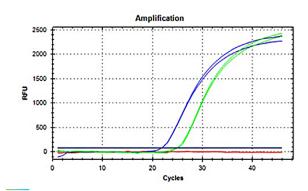


FIGURE 2: DNA extracted from 100 mg ground hazelnut, amplified and detected with qRT-PCR in duplicates (blue curves undiluted, green curves 1:10 dilution)

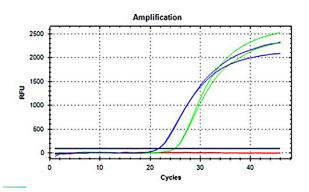


FIGURE 3: DNA extracted from 200 mg ground hazelnut, amplified and detected with qRT-PCR in duplicates (blue curves undiluted, green curves 1:10 dilution).



of lipids are often challenging with respect to extraction as well as to contamination of the extracted DNA with PCR inhibitors. For the extraction of DNA from hazelnut, PCR inhibition can be excluded as ten-fold dilution of the sample. It results in a shift of the ct value of around 3.3 cycles which is the case for extracts from 100 mg and 200 mg, respectively. DNA was extracted from 200 mg cheese as described above. Following extraction, extracts were analyzed by photometry using ScanDrop® 250 and qRT-PCR. qTOWER3 and innuDETECT cheese assay were used for qRT-PCR. Analysis was performed with endpoint method provided by qPCRsoft.

#### CONCLUSION

In summary, the results show that combination of InnuPure® C16 touch and DNA extraction kits for this device can be used for DNA extraction from a multitude of samples related to food industry. DNA extracted with this system has optimum quality. Most important, DNA extracted with InnuPure C16® touch and the relevant kits can readily be used for qRT-PCR.

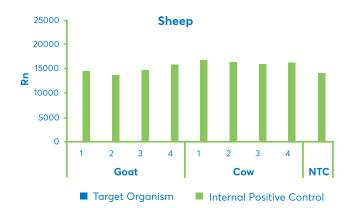


FIGURE 4: Detection of sheep-specific target gene.

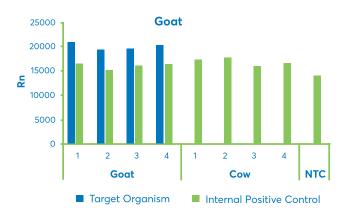


FIGURE 5: Detection of goat-specific target gene

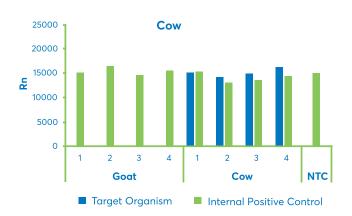


FIGURE 6: Detection of cow-specific target gene.

Cheese type	Replicate	A260/A280	c [ng/µl]
	1	2.03	9.86
	2	2.02	11.07
	3	2.01	12.56
Goat	4	2.01	12.59
	1	2.07	16.3
	2	1.96	12.78
	3	2.03	12.38
Cow	4	2.01	9.84

Table 2: Photometric analysis of nucleic acid extracted from cheese samples.

# Bead Ruptor Elite Bead Mill Homogenizer

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Description	Cat. No.
Bead Ruptor Elite	76000-746
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1.5 mL Tube Carriage Kit	10032-524
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7 mL Tube Carriage Kit	10809-030
30 mL Tube Carriage Kit	10032-384





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Working Well Volume	No. of Wells	Color	Cat. No.
Flat Plates			
200 μΙ	96	Assorted	82006-648





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Description	Volume Range	Cat. No.
Laboratory Automated Workstation, 100/240 V	1–1000 μL	10809-032



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Description	Cell Dimensions	LxWxH	No. of Cells	Cat. No.
Liquid Nitrogen Cryogenic Freezer Boxes with Drain Slots				
2" Cryogenic box with drain slots and grids,				
100 cell divider	1.3 cm (0.5")	13.2 x 13.2 x 5.1 cm (5½ x 5½ x 2")	100	82007-158

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