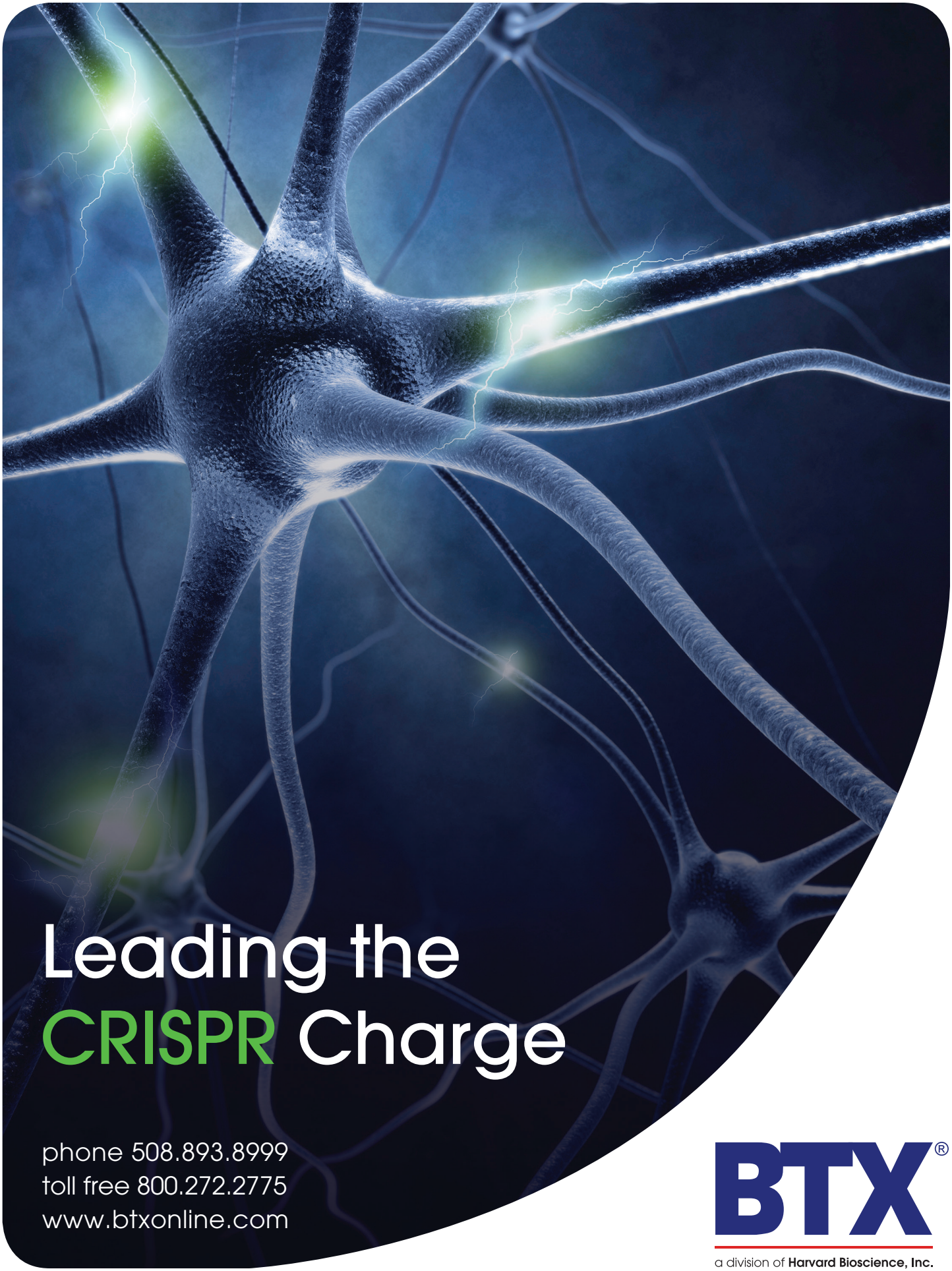


phone 508.893.8999
toll free 800.272.2775
www.btxonline.com

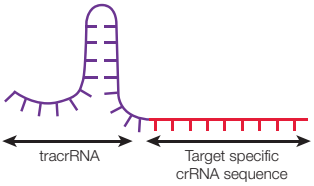
BTX®

a division of **Harvard Bioscience, Inc.**

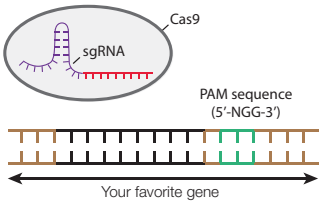
What is CRISPR?

CRISPR, originally discovered as a bacterial 'immune' system against viruses, has been modified for use as a gene editing tool for eukaryotic cells. The system works by creating a complex that includes a piece of RNA that targets a specific DNA sequence (1) and recruits a protein (e.g. Cas9) (2) which cuts the targeted sequence (3). Sequence modifications (e.g. NHEJ Non-Homologous End Joining) (4) to the guide RNA allow researchers to alter genes with unmatched flexibility, precision and efficiency.

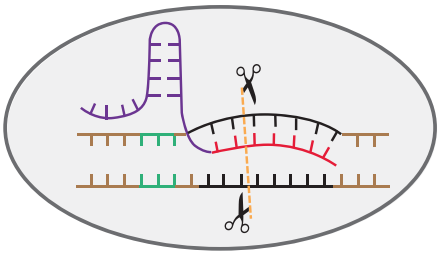
1 **sgRNA (single guide RNA)**



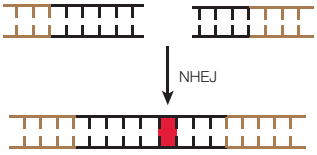
2 **sgRNA + Cas9 protein**

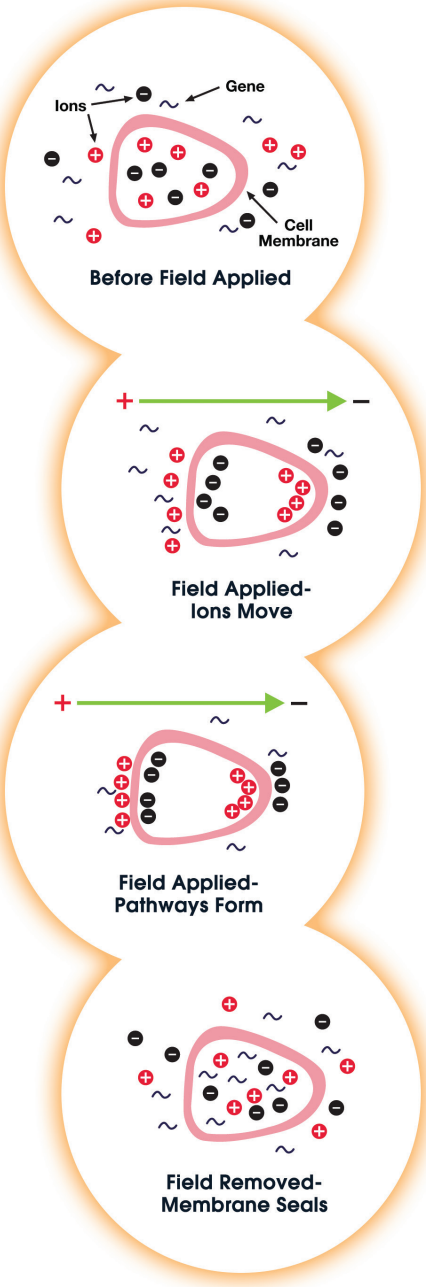


3 **Target specific cleavage**



4 **Cellular error-prone repair "knocks out" gene**





Why Electroporation?

As revolutionary as CRISPR has been, there have been some challenges that have limited its wider application. Efficient transfection of the CRISPR construct into certain cells (e.g. stem cells, neurons, hematopoietic cells, zygotes, etc.) has been difficult if not virtually impossible using standard transfection protocols. Electroporation allows for the efficient transfection of these difficult cell types by inducing transient pores to form in the cell membrane in response to a carefully controlled electrical pulse. The CRISPR construct moves into the cell through these pores and the cell membrane reseals after the pulse.

Feature	Electroporation	Virus	Reagent	Mechanical*
High Efficiency	Yes	Yes**	Yes**	Yes**
Results are Reproducible	Yes	Yes**	Yes**	No
Low Cost per Assay	Yes	No°	No°	Yes
All Cell Types	Yes	No	No	No
All Plasmid Types	Yes	No	No	No
Fast	Yes	No†	No[a]	No
Easy to Use (plug and play)	Yes	No	No	No

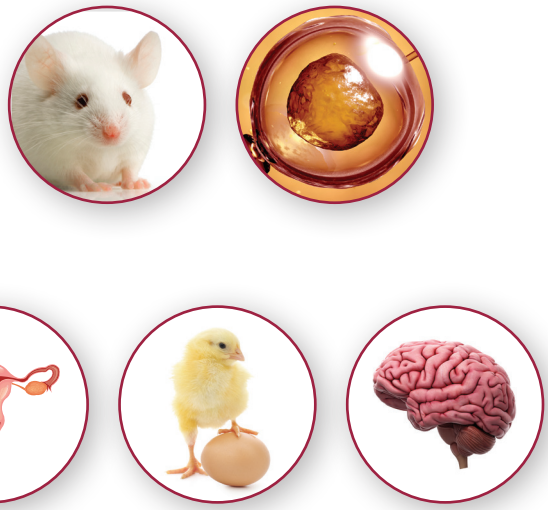
*Mechanical includes Biolistics and Microinjection
** Limited to a few cell types
° Expensive reagents required
† Preparation Time Required
[a] Incubation time required

BTX Electroporation and CRISPR

One of the key success factors in any gene expression and modification experiment (CRISPR, gene editing, engineering) is the optimal and efficient introduction of key components/molecules into your cell line in vitro or in vivo system. Due to its ease of use, reproducibility, high efficiency and low toxicity, BTX electroporation has become the method of choice for introducing CRISPR constructs into cells such as mammalian, bacterial, yeast, plant, parasite and insect.

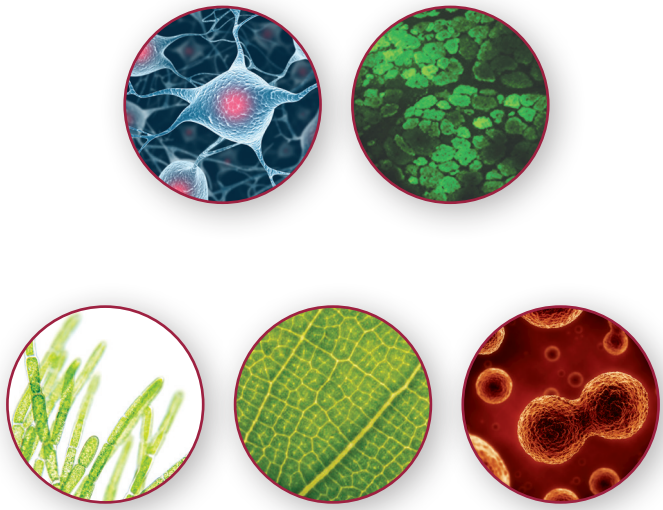
Transfect Everything with CRISPR using BTX systems

In Vivo, Adherent, suspension, and primary cells,
In Utero, In Ovo, Ex Vivo



Electroporate a wide range of cell types including hard to transfect cells with CRISPR using BTX systems

Neurons • Primary Cell Cultures • Immune Cells
Zygotes • Embryos • Parasitic cells



Why BTX?

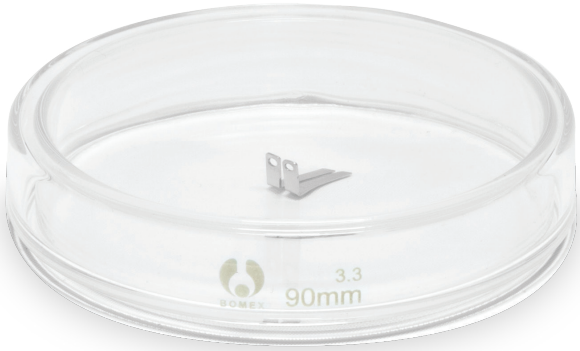
Benefits	Features
Proven and Trusted	Over 10,000 publications from top labs around the world have selected BTX for their work
Guaranteed	Over 30 years with experience of every type of application. Protocols, application notes, and PhD level Technical Support ensures success for the novice to expert user
Easy to Use	Tested pre-optimized protocols bring ‘push button’ success
Easy to Optimize	Automatically saves data that facilitates ideal electroporation setting determination
Customizable	Biggest selection of electrodes and programmability assures a perfect fit with your research
Reproducibility	Storage of all custom protocols ensures consistent results between experiments and users

Oocyte/Embryo Electroporation Made Easy with Oocyte Petri Dish Electrodes

High efficiency, high throughput genome engineering in animal models with CRISPR electroporation of Zygotes using BTX systems

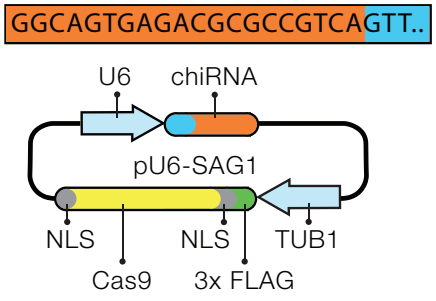
Oocyte electrode:

- Ideal for high throughput mouse genome editing by oocyte, zygoteor embryo electroporation with CRISPR/Cas9 constructs
- Easy to use, fast, high throughput electrode
- Can electroporate 20 – 40 oocytes at a time
- Can visualize embryos during electroporation
- Easy to collect all the embryos after electroporation

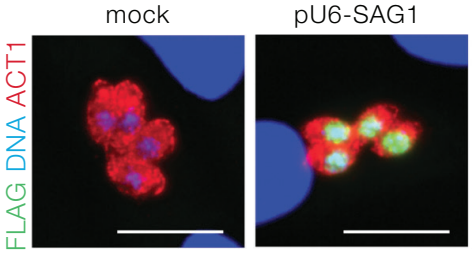


	Electroporation with oocyte Electrode	Electroporation with Cuvette	Micro-injection
Pre-operation set up time	None	None	Preparation time required
Time required for transfecting 100 embryos (time for set up and completion of electroporation /injection)	5 min	5 min	> 2 hours
Throughput	20 – 40 oocytes / run	100 – 200 oocytes / run	1 oocyte / run
Skill required	None	None	Expertise required
Visualization of embryos during transfection	Yes	No	Yes
Cas9 mRNA volume	500 – 2000 ng	500 – 2000 ng	50 – 1000 ng

A



B



Delivery of CRISPR/Cas9 plasmid

into hard to transfect cells such as Toxoplasma gondii using BTX electroporator.

A) CRISPR plasmid (pU6-SAG1) to disrupt SAG1 locus in Taxoplasma gondii cells.

B) Successfully transfected cells (green) as compared to mock transfected cells.

Adapted From: Efficient Genome Engineering of Toxoplasma gondii using CRISPR/Cas9 Sidik SM, et al., 2014 Plos One, volume 9, Issue 6, July 2014

Selection Guide

Find the right Electroporation System and Electrode for CRISPR transfections

Feature	Gemini X2	ECM 830
	All cell and tissue Electroporation	Mammalian cell and tissue Electroporation
Square Waveform	+	+
Multi-Pulsing Square Wave	+	+
Resistance/Pulse Monitoring	+	+
Footswitch Operation	+	+
Exponential Decay Waveform	+	
Multi-Pulsing Exponential Decay	+	
Experiment Log Storage	+	+
Preprogrammed CRISPR and other Protocols	+	+
Unlimited Custom Protocol Storage	+	+
Remote Operation	+	+
PC Communications	+	+

Applications	Cuvette	Plate Handler	In vivo Electrodes
In Vitro (Cuvette)	+		
Eukaryotic Cells	+		
Prokaryotic Cells	+		
In Vivo (Specialty Electrodes)			+
Ex Plant/Tissue Slice (Petri Dish Electrodes)			+
In Ovo (Genetrodes)			+
Adherent Cell (Petri Pulser Electrodes)			+
Multi Well (HT Plate Handler/ 96 Well Plates)		+	

Choose the system you need for CRISPR transfections

Universal System: Gemini X2

In Vitro — All Cell Types — Cuvette/96 Well Applications •
In Vivo • In Utero • In Ovo • Adherent Cell

- Electroporate any cell type — offers both square and exponential decay wave forms
- Touchscreen user interface
- Ease of use with preset protocols including PRESET CRISPR protocols
- Faster optimization with unlimited data logging
- Adjustable for complete user control for easy optimization
- Works with a wide variety of electrodes — cuvettes, in vivo Tweezertrode, Multiwell plate handler

Mammalian Transfection System: ECM 830

In Vitro — Cuvette/96 Well Applications
In Vivo • In Utero • In Ovo • Adherent Cell • Embryos

- Flexible, Workhorse Square wave System
- Well established publication record with CRISPR
- Widest range of voltages available
- Works with a wide variety of Electrodes — cuvettes, in vivo Tweezertrode, Multiwell plate handler

Ordering Information

Part number	Description
Universal System	
45-2041	BTX Gemini X2 Generator only
45-2040	BTX GEMINI X2 ELECTROPORATION SYSTEM: includes Gemini X2 Generator, Cuvettes — 1 mm, 2 mm, 4 mm pack of 30 (10 each), Safety Dome X2, and Cuvette Rack
45-2044	BTX GEMINI X2 HT ELECTROPORATION SYSTEM: includes Gemini X2 Generator, Cuvettes — 1mm, 2 mm, 4 mm pack of 30 (10 each), Safety Dome X2, HT 200 Plate Handler, 1 x 2 mm gap HT Plate, 1 x 4 mm HT Plate and Cuvette Rack
Mammalian System	
45-0662	ECM 830 Generator only
45-2052	ECM 830 ELECTROPORATION SYSTEM: includes 830 Generator, Cuvettes — 1 mm, 2 mm, 4 mm pack of 30 (10 each), Safety Dome, and Cuvette Rack.
45-0664	BTX HT 830 25/200 SYSTEM: includes ECM 830 Generator, 6 x 25-Well 4mm gap HT Plates, & HT-200 Plate Handler
Cuvettes	
45-0124	BTX Electroporation Cuvette, 1 mm Gap, 90 µl, Package of 50, Gray Top, Bacterial
45-0125	BTX Electroporation Cuvette, 2 mm Gap, 400 µl, Package of 50, Blue Top, Bacterial/Mammalian
45-0126	BTX Electroporation Cuvette, 4 mm Gap, 800 µl, Package of 50, Yellow Top, Mammalian
HT Plates	
45-0450	96-Well Disposable Electroporation Plate, 2 mm gap, 125 µl
45-0452	96-Well Disposable Electroporation Plate, 4 mm gap, 250 µl
45-0466	25-Well Disposable Electroporation Plate, 2 mm gap, 125 µl
45-0462	25-Well Disposable Electroporation Plate, 4 mm gap, 250 µl
Reagents	
45-0802	BTXpress Electroporation Solution, 5 ml bottle for up to 50 reactions
45-0805	BTXpress Electroporation Solution, 10 ml bottle for up to 100 reactions
Specialty Electrodes	
45-0160	Genetrode Electrode Kit, 5 mm, Straight. Gold Tip
45-0162	Genetrode Electrode Kit, 5 mm, L-Shaped. Gold Tip
45-0505	Petri Dish Platinum Electrode for Tissue Chamber Kit, 5 mm. Includes Glass Petri Dish with Tissue Chamber 5 mm, Glass Petri Lid
45-0489	Platinum Tweezertrode, 5 mm Diameter, Includes Cables
45-0488	Platinum Tweezertrode, 7 mm Diameter, Includes Cables
45-0494	Platinum Triple Electrode Tweezertrode, 5 mm Diameter, Includes Cables
45-0496	10 mm Oocyte Electrode, Platinum Plated, 1 mm gap, Kit (with cables)
Adherent Cell Electrodes	
45-0130	Petri Pusher, 2 mm gap, gold plated, for 6-well or 35 mm Petri Dish
45-0531	Adherent Cell Electrode, 5mm gap, Kit (with cables)