



PRONOSTICS  
Knowledge for Health



# UltraPlex™

Multiplexing just went digital...

UltraPlex™ from Pronostics - the digital multiplex solution

A complete multiplex solution

ideal for a wide range of applications,

from ELISAs to multiplex PCR,

profiling to high-throughput screens.

Pronostics Ltd. is an ISO 13485:2003 certified company

[www.pro-nostics.com](http://www.pro-nostics.com)

[www.ultraplex.com](http://www.ultraplex.com)

## UltraPlex™ from Pronostics - the digital multiplex solution

---

UltraPlex™ is a complete multiplex solution ideal for a wide range of applications, from ELISAs to multiplex PCR, profiling to high-throughput screens. The possibilities are endless because almost any kind of assay can be readily ported to the microscopic barcoded microparticles at the heart of the UltraPlex™ digital multiplexing system.

The fluorescence read-out from each assay is rapidly decoded on the custom-designed UltraPlex™ SmartReader, running our new SmartDecode software which, thanks to bar-coding protocols with misread protection, essentially guarantees error-free decoding, while generating up to 100,000 data points per hour and being robust and easy to use.

With the dedicated liquid-handling system, the UltraPlex™ SmartStation, we provide a substantial gain in productivity for any application. More data for less effort and lower costs.

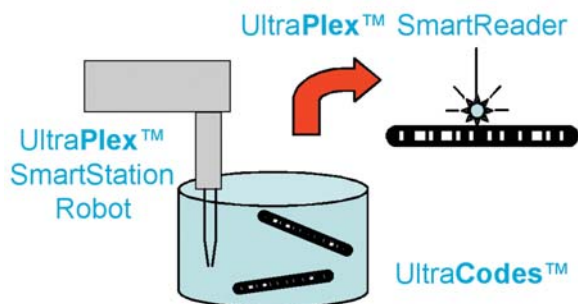
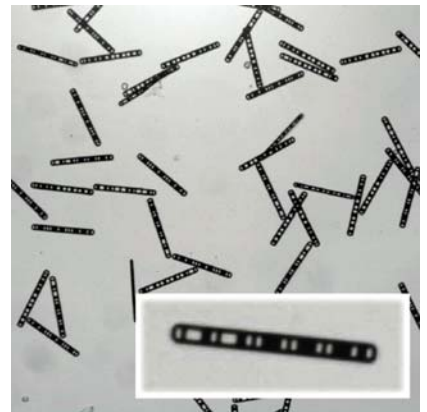


Figure 1



A micrograph of more than 50 five-digit UltraCode™ barcoded microparticles of 10 different codes. Each particle (inset) is only 0.1mm in length.

	5 digit UltraCodes	2 digit UltraCodes
Size (LxWxD; $\mu\text{m}$ )	103 x 10 x 1	45 x 10 x 1
Maximum number of codes	100,000	100
with discontinuous coding	3,235	10
Surface area (typical per particle; $\mu\text{m}^2$ )	1,400	650
Binding capacity ( $\mu\text{g}/\text{cm}^2$ )	1	1
per microparticle (pg)	15	6.5
with biotinylated ligand (pmol)	1	0.45
Stability	> 55°C for 2 hrs; >95°C for 5 min	
to pH	4 – 10	
ionic strength	> 0.2 M NaCl	
Storage	> 3 years	



## UltraCodes

UltraCodes are microscopic aluminium microparticles, each carrying a unique physical barcode. Each microparticle can act as the substrate for a different assay, reaction or test. Mixtures of different UltraCodes can then be mixed together, the assays performed in parallel in a single vessel, and then rapidly decoded by the UltraPlex™ SmartReader.

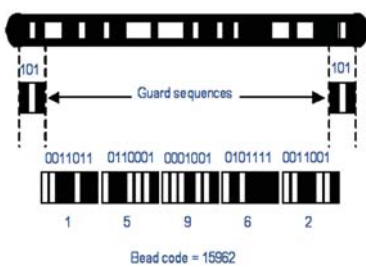
Unlike dye-bead multiplex systems (where the ‘codes’ set by the ratio of fluorescent dyes within the particle are effectively continuous), barcoding brings all the benefits of digital signal quality: there are up to 7 individual components comprising each digit, so a single unreadable component in any one of the digits simply yields an ‘unrecognised’ code which is dismissed by the SmartReader. The result is that mis-identification of a test microparticle is effectively reduced to zero, eliminating one of the major limitations of dye-bead multiplexing. Similarly, because the code is physically etched into the microparticle, it is permanent and cannot bleach like dye-coded beads. Multiplexing with digital detection! (see figure 2)

UltraCodes come in a number of designs, each optimised for different purposes. For many applications, the 5-digit UltraCode microparticle is ideal. Alternatively, where high microparticle densities are required (such as in high multiplex applications), smaller 2-digit UltraCode microparticles accelerate reading times.

One of the major advantages of UltraPlex™ is access to high multiplex (or ‘megaplex’) applications. 5-digit UltraCode microparticles are available in 100,000 different codes. Other UltraCode designs allow even more codes, and we can readily create new custom microparticle designs optimised for particular applications.

Figure 2

Reading the barcode from a 5-digit UltraCode microparticle. Discontinuous coding ensures mis-identity is effectively reduced to zero.



The microparticles are manufactured using conventional semiconductor fabrication techniques, ensuring that very large numbers of microparticles can be produced, exploiting substantial economies of scale. Lot-to-lot variation in properties is tightly controlled, and validated in our extensive ISO 13485:2003 QC process.

UltraCodes are made from aluminium, which does not bind biomolecules directly. As a result, we have developed an array of attachment chemistries which coat the microparticles to create a substrate optimised for biomolecule binding. Microparticles treated using our standard procedure have a similar protein binding capacity to cell-culture grade plastic, such as that commonly used in ELISA wells. However, custom surfaces, optimised for attachment of other molecules, have been developed and are available for use if required.

One straightforward way to attach other biomolecules, such as oligonucleotides or peptides is to employ microparticles coated in streptavidin. Such microparticles can readily bind biotinylated reagent, facilitating high sensitivity detection.

A major advantage of the aluminium substrate is its robust physical properties. UltraCodes survive extremes of pH, ionic strength, organic solvents and high temperature ensuring their compatibility with the widest possible range of analytical methodology. They can be stored for at least 3 years at -20°C, and survive multiple freeze-thaw cycles.

## UltraPlex™ SmartReader

---

UltraCodes can be used as the substrate for almost any assay format that yields a fluorescent output. In principle, it is possible to decode the microparticles and simultaneously measure the fluorescent output using conventional laboratory equipment (such as a fluorescence microscope or flow cytometer). Without dedicated decoding software, however, such manual reading is likely to be impractically slow. As a result, Pronostics have developed a dedicated UltraPlex™ reader, the SmartReader running custom SmartDecode software. *(see figure 3)*

The UltraPlex™ SmartReader is a self-contained unit occupying approximately 0.3 m<sup>2</sup> of bench space, operated through a dedicated PC workstation. Based around a fluorescent microscope, the unit accepts standard 96-well plates and reads up to 100,000 microparticles per hour.

Each well is scanned and microparticles are decoded from a brightfield image, the output of the assay is quantified from a fluorescent image of the same field of view. At least 10 microparticles of each code are measured in each well, and the mean fluorescence reported.



Figure 3

Walk away SmartReader reads up to 100,000 microparticles in just one hour (translating in up to 1000 test results) with SmartDecode software.



The very low intrinsic fluorescence of the aluminium substrate, together with its low protein binding results in a high signal-to-noise ratio (SNR) in many applications. For example, in immunoassays SNR can exceed +30dB (equivalent to a mean signal 25 times background noise), similar to conventional ELISAs. With a reproducibility of 4.9% (coefficient of variation at half of full scale deflection), the UltraPlex™ SmartReader also has reproducibility comparable to conventional absorbance-based ELISA plate readers.

The UltraPlex™ SmartReader is configured with high quality optics to read reporter molecules absorbing at around 595 nm. However, alternative filter sets for other commonly used fluorescent reporters have been validated, and the next generation Reader now under development will allow simultaneous measurement of multiple dyes.

The SmartDecode software outputs the data matrix (which can contain more than 1,500 test results for a 16-plex assay) in a format compatible with all major LIMS systems, or in Excel format for manual analysis, and custom output formats can readily be added.

Available off the shelf, and fully validated, the UltraPlex™ SmartReader is the ideal solution for most medium-throughput applications. However, we have carried out proof-of-principle experiments for high throughput applications decoding UltraPlex™ assays on high-end reader systems in conjunction with collaborative partners, and have shown that data accrual rates 10-fold higher than with UltraPlex™ SmartReader can readily be achieved. Similarly, real time decoding in flow-based readers is also practicable and could be developed further for applications demanding very high throughput.



UltraPlex™ SmartReader

Size (WxDxH)	61 x 67 x 48
Weight	37 kg
Light source	Mercury burner
Optics	Objective UPFL10X Olympus 37542
	Excitation filter 545-580 nm
	Dichroic 600 nm in U-MWIY cube
	Emission filter 610 nm IF bandpass
CCD camera	FirstSight Vision Dolphin 201B
Software	SmartDecode software
Typical scan time (96-well plate)	90 mins
Data accrual (16-plex experiment)	Up to 100,000 barcodes/hr
	Up to 1000 test results/hr
Signal-to-noise	~ +30 dB
Misread rate	< 0.6%
Reproducibility (CV)	4.9% (half full-scale deflection)



# UltraPlex™ SmartStation

The UltraPlex™ system also incorporates a customised liquid-handling robot, the UltraPlex™ SmartStation, which automates assay performance. For example, in clinical diagnostics laboratories 160 samples a day can be subjected to a 11-plex UltraPlex™ assay using a single integrated SmartStation and SmartReader, yielding 1,760 data points in a single day, exceeding the performance of many auto-analysers. (see figure 4)

The standard UltraPlex™ SmartStation robot has a four-tip head, capable of dispensing 20-1000 µl of liquid, and incorporates liquid-level detection technology to ensure reagent transfer. For very high throughput applications the robot is available with a 96-tip head. The system uses disposable plastic tips, but can be reconfigured to re-use tips if required for particular applications. Typically, three washes on a 96-well plate take about 10 minutes to perform.

The SmartStation has four (or more, if required) separate liquid reservoirs suitable for complex protocols with multiple detection reagents, with a dead volume of less than 5 ml conserving costly assay components.

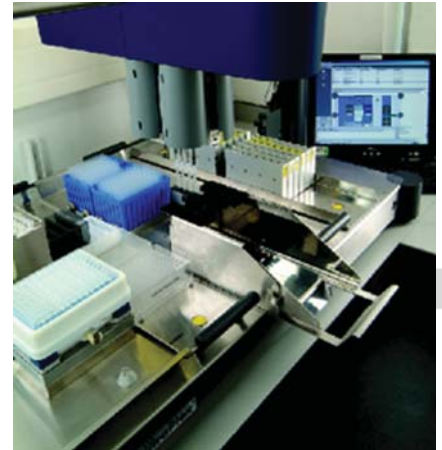
Importantly, the system has a shaking vacuum manifold optimised for handling UltraCodes in conventional filter plates. Microparticle losses are minimised, and the results obtained from an automated assay are at least equivalent to the manual performance of the same assay.

Samples can be taken from a wide range of tubes (1.5 to 10 ml volume) or from 96-well plates. The UltraPlex™ SmartStation also incorporates a standard barcode reader (compatible with most standard barcode formats) for sample tube identification. This ensures that the sample ID and data subsequently obtained from it are permanently linked, effectively eliminating sample-handling errors.

The SmartStation is driven by an integrated PC workstation, running Lirix™ software†. In addition to delivering ‘one-button’ convenience to operate standard protocols, it also offers the user immense flexibility in designing new protocols for a wide range of applications.

† Lirix software is provided under license from Xiril, A.G.

Figure 4



The UltraPlex™ SmartStation liquid handling system automates assay performance, and directs data into local LIMS systems, increasing throughput, improving replicate reproducibility and eliminating data transcription errors.

## UltraPlex™ SmartStation

Size (WxDxH; cm)	106 x 70 x 72
Weight	80 Kg
Reservoirs	300ml, 40ml or 2ml tubes
Dead volume	10ml, 4ml or 40µl
Head	4-tip
Re-use tip racks	Optional
Vacuum manifold	Upto 850mbar; 750rpm
Software	Lirix Version 2.2.22†
Wash time (96-well plate; typical)	210 secs
Cycle time (typical immunoassay)	~3 hours
Operating noise level	< 70dB

## Applications

### Direct Immunoassays

Pronostics have developed and launched a range of immunoassay products for immunology laboratories in the autoimmune area, including UltraPlex™ ANA and UltraPlex™ Thyroid which have detection sensitivities at least as good as conventional direct immunoassays. Direct immunoassays for allergen-specific IgEs detect < 1 IU/ml (about 3-fold higher than commercial uniplex assays) without any optimisation.

**Sandwich ELISAs** Proof-of-principle multiplexed sandwich ELISAs for cytokines using UltraPlex™ achieve sensitivities below 10 pg/ml, comparable to dye-bead multiplex cytokine ELISA products, and sufficient for most applications.

**DNA hybridisation** Proof-of-principle studies demonstrate high-specificity hybridisation to biotin-oligonucleotides immobilised on streptavidin-coated UltraCodes.

**Activity assays** Active enzymes can be coupled onto UltraCodes, either directly or via streptavidin-biotin. Binding of fluorescent substrates (or the modulation of substrate binding using an unlabelled library) can then be readily quantified.

### Receptor binding assays

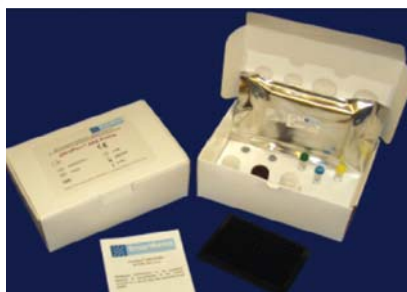
Where functional receptors can be isolated fluorescent ligand binding can form the basis of a multiplexed high-throughput screening paradigm. With multicolour detection this underpins a '3D screening' framework which will revolutionise drug discovery.

**Cell-based assays** Live cells can also be grown on UltraCode microparticles, allowing functional assays (such as cell-cycle regulation, control of apoptosis and so forth) to be multiplexed. For many receptors, whole cell assays are also the only practical route to high-throughput screening molecular screening.



Figure 5

The UltraPlex™ ANA assay kit from Pronostics provides 9 semi-quantitative tests for different anti-nuclear antibodies and a quantitative test for anti-dsDNA auto-antibodies, in a convenient product format for the UltraPlex™ system.



The UltraPlex™ system has already been used to multiplex a wide range of assays that demonstrate its broad applicability.

The most advanced applications are in direct immunoassay, where Pronostics have already developed and launched a series of products directed at clinical immunology laboratories. Importantly, these products demonstrate that UltraPlex™ assays yield sensitivities and specificities at least as good as conventional "one analyte at a time" ELISAs. (see figure 5)

We continue to develop new products based on direct immunoassays, ranging from allergen-specific IgE assays, to a unique class of profiling diagnostics that use a 64-plex profile of the immune system to diagnose coronary heart disease.

Sandwich ELISAs can be ported to UltraPlex™ as readily as direct immunoassays: for example, a panel of cytokine assays yielded sensitivities around 10 pg/ml comparable to conventional competing products.

Applications are not limited to immunoassays. We have demonstrated that biotinylated oligonucleotides can be readily coupled to streptavidin-coated UltraCodes, and then specifically hybridized with labelled probes, opening up a wide range of potential applications in multiplex genetic testing.

Similarly, active enzymes (such as proteases), receptors or even live cells can be immobilised on UltraCodes facilitating multiplex high throughput screening approaches where a library can simultaneously be tested for activity against a wide range of targets.





Pronostics Ltd.  
Babraham Hall  
Babraham  
Cambridge

CB22 3AT

UK

+44 (0)1223 496 730

[sales@pro-nostics.com](mailto:sales@pro-nostics.com)

Pronostics Ltd. is an ISO 13485:2003 certified company

[www.pro-nostics.com](http://www.pro-nostics.com)

[www.ultraplex.com](http://www.ultraplex.com)

Version 1.0 29/4/08