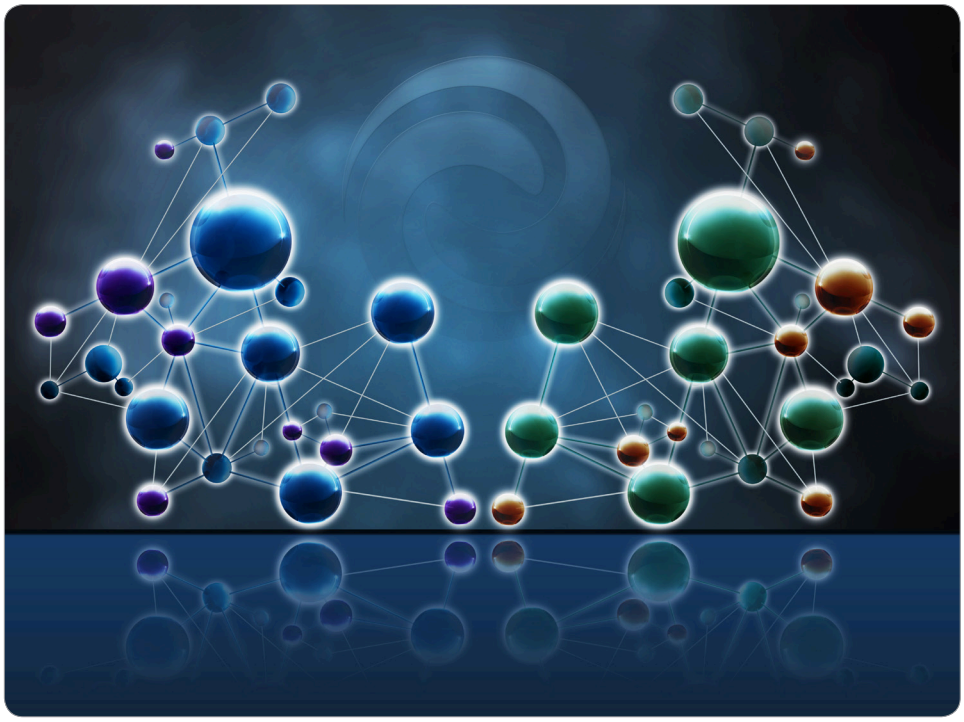
nCounter®
Plex²™ Expression Assay



Product Highlights

- **Multiplex in 2 dimensions:** multiplex hundreds of targets and multiple samples (up to 4) in a single tube
- **Customizable to study size:** measure between 20–400 customizable targets in a single tube
- **Superior precision compared to qPCR:** no replicate data points required
- **Directly assay tissue, blood lysates, and FFPE extracts** in a simple workflow
- **Analyze up to 384 samples*** per day

nCounter Plex² Expression Assay Overview

The NanoString **nCounter Plex Expression Assay** increases sample throughput by utilizing the nCounter Analysis System's unique barcode technology to multiplex in two dimensions, both samples and targets in a single lane.

The Plex² Expression Assay Kit is available to researchers that want to increase the throughput of their nCounter System to up to 384* samples per day in CodeSets of up to 200 targets or 192 samples per day, for up to 400 targets. All nCounter assays deliver direct digital detection, rather than relative fluorescence, thus providing superior reproducibility. The Plex² Expression assay provides researchers with this same high-resolution result in a new high-throughput format.

The Plex² kits arrive in your lab with the highly-multiplexed CodeSet and all the reagents and consumable necessary to perform the assay. Kits are available in sample sizes of 768 and above and offer a high-quality data in a high-throughput format for large study sizes.

** requires two prep stations.*

Flexible Sample and Target Study Configurations

The nCounter system's ability to multiplex both samples and targets provides flexibility in study configurations depending on the throughput required and the amount of genes to be interrogated. **TABLE 1** below displays the number of samples that can be profiled in a single tube with the number of targets that can be profiled along with the amount of initial sample required. Additionally, it displays the number of samples that can be profiled within a single day and the number of liquid handling steps required for each sample.

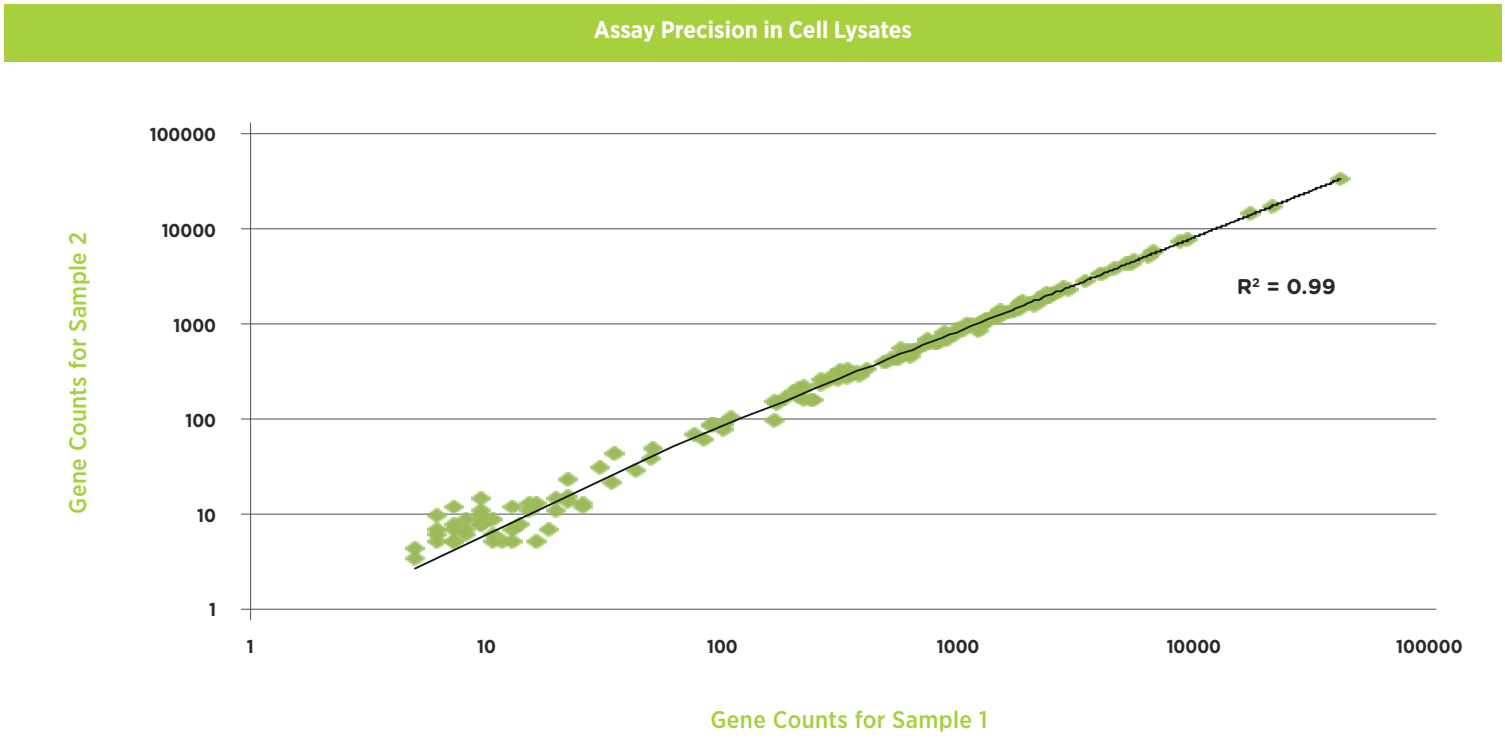
For studies with high numbers of samples, the Plex² assay can be used to measure up to four samples in a single well and 200 targets can be profiled. For studies where a high number of targets is required (up to 800) a single sample can be added to a well using the standard nCounter Gene Expression Assay.

TABLE 1 Flexible sample and target multiplexing configurations

# Samples per Well	# Targets Profiled per Sample	Amount of RNA required per Sample	Sample Throughput per Day	# Liquid Transfer Steps per Sample
1 sample – Standard Gene Expression Assay	**800 targets**	**100 ng**	**96**	**4**
2 samples – Plex² Assay	**400 targets**	**100 ng**	**192**	**5**
4 samples – Plex² Assay	**200 targets**	**150 ng**	**384**	**5**

To determine the precision of the assay when 4 samples are profiled in a single tube using cell lysates, multiplexed single tube hybridizations of 200 probe CodeSets and 4 samples derived from 2.5 µl of Hela cell lysates were processed using the nCounter system. Technical replicate data were normalized and gene expression counts were then plotted to determine assay precision across all replicates (**FIGURE 1**). The plot below displays the correlation of 2 replicate samples. Comparisons between replicates showed $R^2 \geq 0.99$.

FIGURE 1 Multiplexed single tube hybridization of 200 probe CodeSets and 4 samples derived from cell lysates to determine precision across all samples.



High-throughput Benefits for Studies with Large Numbers of Samples

Sample Multiplex	# of Genes per Run		Samples per Day		Data Points per Day
Standard (1 - Plex)	**800** genes	X	**96** samples	=	**76,800***
nCounter Plex² (2 samples per lane)	**400** genes	X	**192** samples	=	**76,800***
nCounter Plex² (4 samples per lane)	**200** genes	X	**384** samples	=	**76,800***

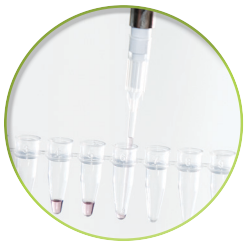
** Requires 2 Prep Stations*

nCounter Analysis System Overview

The **nCounter Analysis System** from NanoString offers a cost-effective way to easily profile hundreds of gene transcripts simultaneously with high sensitivity and precision. The digital detection of target molecules and high levels of multiplexing eliminate the compromise between data quality and data quantity, bringing better sensitivity, reproducibility, and linearity to your results. It is ideal for studying defined gene sets across a large sample set, *e.g.*, microarray validation or NGS, pathway analysis, biomarker validation, and splice variation analysis.

The system utilizes a novel digital technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity (<1 copy per cell). The technology uses molecular "barcodes" and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction.

Simple, User-friendly Workflow

	1	2	3
	Hybridization	**Sample Processing**	**Digital Data Acquisition**
Only 15 Minutes of Total Hands-on Time			
Process	**Set Up Hybridization** Add buffer, CodeSet and sample into a strip tube and hybridize overnight.	**Set Up Prep Station** Place the strip tube onto the automated nCounter Prep Station with reagents and consumables from the nCounter Master Kit.	**Set Up Digital Analyzer** Take the cartridge from the Prep Station and place it into the Digital Analyzer for direct digital counting.
Hands-on Time	5 minutes	5 minutes	5 minutes
	Day 1	**Day 2** (automated)	**Day 2** (automated)

System Performance

Description	Specifications
Level of multiplexing	Up to 200 gene targets for 4 samples per cartridge lane Up to 400 gene targets for 2 samples per cartridge lane
Recommended amount of starting material	150 ng for 4 samples per cartridge lane
Sample types supported	Total RNA, cell lysates in GITC, FFPE-derived total RNA and PAXgene™ lysed whole blood
Fold change sensitivity	≥ 2-fold change (≥ 1 copy per cell)
Spike correlation	$R^2 \geq 0.95$
Linear dynamic range	7×10^5 total counts
nCounter Prep Station throughput with Plex² Assay Kit	48 samples / 2.5 hours
nCounter Digital Analyzer throughput with Plex² Assay Kit	48 samples / 2.7 hours (up to 384* samples per day unattended running per day)
Controls	6 positive assay controls 8 negative assay controls

** Requires 2 Prep Stations*

Ordering Information

Description	Quantity / Use	Part Number (P/N)
nCounter Plex² Expression Assay Kit - 2 samples per lane	768 assays 960 assays 1536 assays 2304 assays	XT-GXA-2PLX-768; XT-GXA-2PLX-960; XT-GXA-2PLX-1152; XT-GXA-2PLX-1344; XT-GXA-2PLX-1536; XT-GXA-2PLX-1920; XT-GXA-2PLX-2304;
nCounter Plex² Expression Assay Kit - 4 samples per lane	768 assays 960 assays 1536 assays 2304 assays	XT-GXA-4PLX-768; XT-GXA-4PLX-960; XT-GXA-4PLX-1152; XT-GXA-4PLX-1344; XT-GXA-4PLX-1536; XT-GXA-4PLX-1920; XT-GXA-4PLX-2304;
nCounter Analysis System *(includes the Prep Station and Digital Analyzer)*	1	NCT-SYS-120
Additional nCounter Prep Station	1	NCT-PREP-120
Additional nCounter Digital Analyzer	1	NCT-DIGA-120

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