Defining a QuantiGene Plex Assay panel

This document provides information to help you define an Invitrogen™ QuantiGene™ Plex Assay panel for your particular needs. It includes:

- Specifications for QuantiGene Plex Assays
- Recommendations for selecting housekeeping genes
- Requirements for ordering a QuantiGene Plex panel
- Housekeeping genes available for use in QuantiGene Plex Assays

QuantiGene Plex Assay specifications

Table 1. QuantiGene Plex Assay performance specifications.

Parameter	Range
Limit of detection	<1,000–2,000 transcripts/assay well
Limit of quantitation	<2,000–4,000 transcripts/assay well
Linear dynamic range	>3 logarithmic units
Assay precision (CV)	<15% intra-assay, <20% inter-assay
Compatible sample types	Cultured cells, bacteria, whole blood, PAXgene™ blood tube or dried blood spots, fresh or frozen tissue (animal or plant), formalin-fixed, paraffin-embedded (FFPE) tissue, purified RNA
Assay format	96-well or 384-well plates
Targets/well	3–80

Selecting housekeeping genes

Every panel should include 3–5 housekeeping genes. The purpose is to enable the following:

- Flexibility in data analysis, in case a selected housekeeping gene for a specific project does not behave as expected
- Ability to normalize the data using a geometric mean of several housekeeping genes' values. This technique "smooths" any bias from a single housekeeping gene
- Provide alternatives in case the signal from one housekeeping gene is too low or saturating
- Flexibility for use for different sample types (cultured cells, blood, tissues, RNA)

Try to select housekeeping genes that are expressed:

- Consistently, under all experimental conditions being evaluated
- At levels similar to those of your target genes, so that a single sample input yields a linear response for all targets

Essential information to include in an order

Before you place an order for a QuantiGene Plex panel, make sure you have the following:

- Unique identifier for each target gene, preferably an accession number and version or GI number; if neither of these is available, submit the exact nucleotide sequence of the transcripts you wish to detect
- 3–5 housekeeping RNAs in each panel (highly recommended)
- Available information on relative expression of target and housekeeping RNAs in your experimental system, especially RNAs of low abundance
- If data from another gene expression platform are to be compared to results from a QuantiGene Plex panel, provide sequences of primers or probes used in that platform; we will design probe sets to these regions whenever possible
- If the panel is for validating RNAi knockdown, you may provide the region(s) targeted by the siRNA; we will build the probe sets centered on these regions whenever possible

- Additional specificity requirements—for example:
  - You wish to distinguish RNAs from human cells transplanted into a mouse model. In this case, we will design probe sets to be specific to the human RNA of interest but not the mouse homolog, and it will not cross-react with any other human or mouse RNAs.
  - You wish to distinguish splice variants. In this case, provide both the exact sequence you wish to detect and the sequences of variants you do not wish to detect.

Note: Unless otherwise specified, probe sets are specific to the RNA sequence submitted or referred to by accession.version or GI number and will not cross-react with any other RNAs (unless otherwise noted on the product insert) in the genome of the organism from which that RNA sequence is derived.

Relative expression of some housekeeping genes

The following table provides general guidelines for the linear assay working range based on the number of cells per assay well:

Table 2. Common housekeeping genes and expression levels.

Relative expression	Housekeeping genes	Guidelines for number of cells per assay well
High	*ACTB*, *B2M*, *GAPDH*, *PPIA*, *RPL19*, *RPS23*, *RPL32*, *RPLR0*, *RPS3*, *RPS18*, *RPS20*	50–50,000
Medium high	*LDHA*, *PGK1*, *PPIB*, *RPL13A*, *UBC*	100–30,000
Medium	*HPRT*, *POLR2A*, *TFRC*	400–60,000
Low	*ATP6V1A*, *GUSB*, *HMBS*, *TBP*, *TNX2*	2,000–80,000

Recommended housekeeping genes for various sample types

The following table provides recommendations for housekeeping genes, based on the type of sample you are examining.

Table 3. Housekeeping genes for various sample types. +: not recommended, ++: fair, +++: good, ++++: best.

Relative expression	Housekeeping genes	Whole blood or PAXGene tube*	Cultured cells (20,000 cells/well)	Fresh or frozen tissue*	Purified RNA (250 ng total RNA)	FFPE tissue*
High	*ACTB*, *B2M*, *GAPDH*, *PPIA*, *RPL19*, *RPS23*, *RPL32*, *RPLR0*, *RPS3*, *RPS18*, *RPS20*	+++	+	++	+	++
Medium high	*LDHA*, *PGK1*, *PPIB*, *RPL13A*, *UBC*	++++	+++	+++	+++	++++
Medium	*HPRT*, *POL2A*, *TFRC*	++	++++	++++	++++	++++
Low	*ATP6V1A*, *GUSB*, *HMBS*, *TBP*, *TNX2*	+	+++	++	+++	+

* Using maximal sample input.

Common housekeeping genes

The following table provides full names and accession numbers of the housekeeping genes listed in the previous tables.

Table 4. Common housekeeping genes.

Symbol	Name	Accession number, human	Accession number, mouse	Accession number, rat
ACTB	Actin, beta	NM_001101	NM_7393	NM_031144
ATP6V1A	ATPase, H^+ transporting, lysosomal 70 kDa, V1 subunit A	NM_001690	NM_007508	XM_001058034
B2M	Beta-2-microglobulin	NM_004048	NM_009735	NM_012512
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	NM_008084	NM_017008
GUSB	Glucuronidase, beta	NM_000181	NM_010368	NM_017015
HMBS	Hydroxymethylbilane synthase	NM_000190	NM_013551	NM_013168
HPRT1	Hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	NM_000194	NM_013556	NM_012583
LDHA	Lactate dehydrogenase A	NM_005566	NM_010699	NM_017025
PGK1	Phosphoglycerate kinase 1	NM_000291	NM_008828	NM_053291
POLR2A	RNA polymerase II (DNA directed) polypeptide A	NM_000937	NM_009089	XM_343922
PPIA	Peptidylprolyl isomerase A (cyclophilin A)	NM_21130	NM_008907	NM_017101
PPIB	Peptidylprolyl isomerase B (cyclophilin B)	NM_000942	NM_011149	NM_022536
RPL13A	Ribosomal protein L13A	NM_012423	NM_009438	NM_173340
RPL19	Ribosomal protein L19	NM_000981	XM_001476576	NM_031103
RPL32	Ribosomal protein L32	NM_000994	NM_171086	NM_013226
RPLP0/Arbp	Ribosomal protein, large, P0 (human) Acidic ribosomal protein P0 (mouse, rat)	NM_001002	NM_007475	NM_0022402
RPS3	Ribosomal protein S3	NM_001005	NM_012052	NM_001009239
RPS18	Ribosomal protein S18	NM_022551	NM_011296	NM_213557
RPS20	Ribosomal protein S20	NM_001023	NM_026147	NM_001007603
RPS23	Ribosomal protein S23	NM_001025	NM_024175	NM_078617
RPS29	Ribosomal protein S29	NM_001032	NM_009093	NM_012876
TBP	TATA box binding protein	NM_003194	NM_013684	NM_001004198
TFRC	Transferrin receptor (p90, CD71)	NM_003234	NM_011638	XM_340999
TXN2	Thioredoxin 2	NM_012473	NM_019913	NM_053331

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