2100 Bioanalyzer

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Senior Application Scientist
The Lab-on-a-Chip Approach

Increasing quality and speed of gel electrophoresis

Sample volumes 1 - 5 µl
10 -12 samples depending on Assay
Separation, staining, detection of samples
Results in 5-30 minutes available
No extra waste removal needed
Disposable Chip, no crosscontamination
Three Chip Types

DNA/RNA analysis  Protein analysis  Cell analysis

Agilent Technologies
Agilent 2100 Bioanalyzer

- Exchangeable cartridge for different assays
- Optics for detection
- 16 pin electrodes connected to HV-sources
- Chip holder with heater plate

2. Run Analysis
Current 2100 Analysis Kits

Electrophoretic Separations

**DNA Assays:**
1000, 7500, 12000
- Sizing
- Quantitation
- PCR products, digests, larger DNA fragments
- 12 samples in 30 min.

**RNA Assays:**
nano, pico, Small RNA
- Quantitation (Sizing in Small RNA)
- total RNA, mRNA
- purity & integrity determination
- 10 samples in 30 min.

**Protein Assays:**
P80, P230, HSP-250
- Sizing
- Quantitation
- cell lysates, column fractions, purified proteins, antibodies etc.
- 10 samples in 40 min.

Flow Cytometry

**Cell Assays:**
Flexible use
- Analysis of 6 samples
- Two color detection
- Analysis of protein expression in cells
Cell Applications

- Protein Expression
- Apoptosis Detection
- Gene Silencing
- Transfection Monitoring
- Cell Staining Inside / outside

Video
Cell Assay

Apoptosis

Transfection Efficiency Monitoring
• Detection of GFP-transfected cells
• Antibody staining: Detection of transfected cells expressing the encoded protein

Protein Expression Monitoring
• Extracellular and Intracellular Antibody staining for detection of protein expressed on the cell surface, in the cytoplasm, or in the nucleus

Gene silencing
Principle of Pressure-Driven Flow

For cell assays (analysis of cell fluorescence parameters)
On-chip simple flow cytometric studies

Pressure driven flow is used to move cells in a controlled manner through the micro-channels

Cells are hydrodynamically focused to a portion of the channel by a side stream of buffer

Cells pass the fluorescence detector in single file and each event is monitored in a histogram or dot plot

The micro-channels of the glass chip are filled with cell buffer
The Bioanalyzer Lab-on-a-Chip Approach

**Separation on disposable, μ-fabricated glass chips**
- made of two glass layers:
  - one with micro-channels (x10μm, etched),
  - one with through-holes
- glued into a plastic caddy which accommodates wells for gel, sample, standard (ladder), buffer and other reagents
- for handling nl-amounts of liquids
- one separation channel for ladder and sample
- microfluidic sample movement with fluorescence detection

**Setup**
- micro-channels are filled with gel or buffer
- sample, ladder and reagents are filled into the respective wells
- chip preparation in less than 5 minutes

**Benefits**
- convenient handling
- minimized risk of cross-contamination
- versatile design for multiple experiments on one platform
Flow Cytometry on a Chip - Hydrodynamic Focusing

All six cell samples are hydrodynamically focused to one side of the micro channel.

At each of the six pinch points the cell stream is joined by a buffer stream from one of the two buffer wells.

The two liquids do not mix immediately.

The cells then move towards the detector in single file.
Flow Cytometry on a Chip
- Two Color Detection- Three Types of Events

Dot plot view for easy data evaluation
Some Target applications

Apoptosis:

- **Annexin V**: Detection of phosphatidylserine on the cell surface
- **Caspase-3**: Detection of activated caspase-3 in the cytoplasm

Transfection Efficiency Monitoring:

- **GFP**: Detection of GFP-transfected cells
- **Antibody staining**: Detection of transfected cells expressing the encoded protein

Protein Expression Monitoring:

- Extracellular and Intracellular Antibody staining for detection of protein expressed on the cell surface, in the cytoplasm, or in the nucleus

Gene silencing:

- Optimization of siRNA transfection procedure
- Verify silencing by cellular protein expression measurement
- Correlation of siRNA uptake and gene knockdown
Cell assays: sample preparation

Typical workflow:

- **Customer**
  - Adherent
  - Suspension
  - (Trypsinize) & harvest by centrifugation, wash

- **Agilent**
  - Add staining reagent and incubate
  - Wash twice
  - Resuspend cells in cell buffer (LabChip Kit)
  - Load on chip
  - Data analysis to result
Flow Cytometry on a Chip - Optics & Detection

2100 Bioanalyzer

Red detection channel:
• 620-645 nm excitation with Laser (Maximum 630 nm)
• 674-696 nm detection range (Maximum 680 nm)

Blue detection channel:
• 458-482 nm excitation with LED (Maximum 470 nm)
• 510-540 nm detection range (Maximum 525 nm)
Cell Assays - Applications: Apoptosis

Annexin Binding

Healthy cell

Dead cell

"Live" apoptotic cell

Live dye: Calcein

biotin-Annexin+ Cy5-streptavidin

Phosphatidyl-serine from inner leaflet flips to outer membrane during apoptosis and can be labeled by Annexin V
Annexin V Assay (24h Induction)

Three Bioanalyzer instruments vs a flow cytometer reference instrument

5 chips, each loaded with control in well 1 and 24h sample in wells 2-6

% Apoptotic

Chip 1  Chip 2  Chip 3  Chip 4  Chip 5

Sample Number

0  10  20  30

0%  20%  40%  60%  80%

2100-1  2100-2  2100-3

Flow cytometer
Applications: Protein Expression Analysis
GFP Transfection Efficiency Control

CHO-K1 cells were transfected with EGFP DNA and Lipofectamine.

Control

EGFP transfected
GFP Transfection Efficiency

<table>
<thead>
<tr>
<th>Chip Number</th>
<th>2100-1</th>
<th>2100-2</th>
<th>2100-3</th>
<th>All</th>
<th>Flow cyt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ctrl mean</td>
<td>0.46</td>
<td>0.31</td>
<td>0.47</td>
<td>0.40</td>
<td>0.16</td>
</tr>
<tr>
<td>SD</td>
<td>0.08</td>
<td>0.29</td>
<td>0.43</td>
<td>0.29</td>
<td>0.12</td>
</tr>
<tr>
<td>GFP mean</td>
<td>60.19</td>
<td>59.15</td>
<td>59.26</td>
<td>59.53</td>
<td>60.90</td>
</tr>
<tr>
<td>SD</td>
<td>2.13</td>
<td>2.48</td>
<td>2.10</td>
<td>2.26</td>
<td>1.22</td>
</tr>
<tr>
<td>%CV</td>
<td>3.54</td>
<td>4.19</td>
<td>3.54</td>
<td>3.80</td>
<td>2.01</td>
</tr>
</tbody>
</table>
Flow Cytometry Assays Applications - Cell surface Antibody staining

Cell expressing protein of interest

Cell not expressing protein of interest

Target protein

Live dye: Calcein
Cy5 or APC-labeled Antibody
Extracellular Antibody Staining

Jurkat cells were stained with calcein alone or with calcein and APC-labeled anti-CD3 antibody. Mixtures of both populations were prepared at various ratios. Samples were analyzed with four 2100 instruments on 5 chips and compared to a flow cytometer reference instrument.
GFP On-Chip Staining - Workflow

**Conventional**

- 15 min
- CBNF
- 10 min
- Resuspend cells in CB
- 10 min
- Spin, aspirate, resuspend, spin, aspirate

**On-chip**

- 15 min
- cells + CBNF
- 15 min
- CBNF

35 min
GFP On-Chip Staining - Histogram Quality

2100 bioanalyzer

Flow Cytometer
Lab-on-a-Chip - Principle of Injection & Separation

1. Separation channel
2. Fill
3. Fill
4. Dispense 40 picoliters

Direction of electrodrevn movement of liquids and molecules within liquids
The LabChip® Approach - Simplified Model
(see chip animation.ppt)
DNA Applications

- mPCR validation, impurity check
- Gene Expression
- Restriction Digest Analysis
- Food Analysis
- Forensic Testing
Application Areas for the DNA Assays

PCR product purity

Multiplex PCR Applications

Gene expression analysis via RT-PCR (target validation)

GMO testing

Pathogen detection (homeland defense, hospitals, environmental)

Genotyping applications
  • Duplications/ deletions
  • Allele frequency
  • Bacterial sub-typing
  • Forensics

Cancer diagnostics
<table>
<thead>
<tr>
<th></th>
<th>DNA 1000 Assay</th>
<th>DNA 7500 Assay</th>
<th>DNA 12000 Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sizing range</strong></td>
<td>25–1000 bp</td>
<td>100–7500 bp</td>
<td>100–12000 bp</td>
</tr>
<tr>
<td><strong>Sizing resolution</strong></td>
<td>25–100 bp: 5 bp</td>
<td>100–500 bp: 5 % CV</td>
<td>100–1000 bp: 5 %</td>
</tr>
<tr>
<td></td>
<td>500–1000 bp: 10 % CV</td>
<td>1000–7500 bp: 15 %</td>
<td>1000–12000 bp: 10 %</td>
</tr>
<tr>
<td><strong>Sizing accuracy</strong></td>
<td>± 10 %</td>
<td>± 10 % CV *</td>
<td>±15 %</td>
</tr>
<tr>
<td><strong>Sizing reproducibility</strong></td>
<td>5% CV *</td>
<td>5% CV *</td>
<td>5% CV *</td>
</tr>
<tr>
<td><strong>Quantitation accuracy</strong></td>
<td>20% CV *</td>
<td>20% CV *</td>
<td>25% CV *</td>
</tr>
<tr>
<td><strong>Quantitation reproducibility</strong></td>
<td>25–500 bp: 15 % CV *</td>
<td>100–1000 bp: 10 % CV</td>
<td>100–1000 bp: 15 % CV*</td>
</tr>
<tr>
<td></td>
<td>500–1000 bp: 5 % CV *</td>
<td>1000–7500 bp: 5 % CV*</td>
<td>1000–12000 bp: 10 % CV*</td>
</tr>
<tr>
<td><strong>Quantitative range</strong></td>
<td>0.1 - 50 ng/µL *</td>
<td>0.1 - 50 ng/µL *</td>
<td>0.1 - 50 ng/µL *</td>
</tr>
<tr>
<td><strong>Maximum salt concentration in sample</strong></td>
<td>250 mM for KCl, 15 mM for MgCl₂ 250 mM NaCl</td>
<td>250 mM for KCl, 15 mM for MgCl₂ 250 mM NaCl</td>
<td>250 mM for KCl, 15 mM for MgCl₂ 250 mM NaCl</td>
</tr>
<tr>
<td><strong>25 chips per kit</strong></td>
<td>DNA 12/chip = 300 samples/kil</td>
<td>DNA 12/chip = 300 samples/kil</td>
<td>DNA 12/chip = 300 samples/kil</td>
</tr>
</tbody>
</table>

* Respective DNA ladder as sample
Data Format - Gel-Like Image c/w Agarose Gel

2100 bioanalyzer data
Gel-like image

2 % agarose gel stained with Ethidiumbromide
Determination of PCR Product Impurity
GMO Detection: Determination of GM Soya Percentage

EPSPS gene target: specific for Roundup Ready GM soya (Monsanto)
Optimization of Multiplex PCR on a 19-plex PCR

Data kindly provided by QIAGEN GmbH, Germany
Tumor Diagnostics

1. Spiking experiment with given amount of cancer cells
2. Enrichment with AdneGen Cancer Select kit (antibody based immunomagnetic enrichment.)
3. Multiplex Amplification with AdnaGen CancerDetect kit
4. Detection with Agilent 2100 Bioanalyzer and DNA 500 LabChip kit

Data kindly provided by Adnagen
Detection of Single Base Mutations (1)
in Exons 7 and 8 of the Human p53 Gene by RFLP Mapping using the DNA 7500 kit

Amplify exons 7 and 8 (resulting products: 618 bp fragment and 200 bp fragment)

Digest with Hpa II

In each example one of the restriction sites can be deleted by a point mutation

Analyze using Agilent 2100 bioanalyzer and 4-20 % acrylamide gel
Detection of Single Base Mutations (2)

**p53Exon 8 wt/HpaII**

- **Lower Marker**: 90 bp, 111 bp, 208 bp
- **Upper Marker**

**Fluorescence vs. Time (seconds)**

- **p53Exon 8 clone 106/HpaII**

- **Lower Marker**: 109 bp, 91 bp

**Fluorescence vs. Time (seconds)**

**p53Exon 7 wt/HpaII**

- **Lower Marker**: 84/85 bp, 166 bp
- **Upper Marker**: 267/268 bp

**Fluorescence vs. Time (seconds)**

**p53Exon 7 clone 59/HpaII**

- **Lower Marker**: 276 bp, 168 bp, 91+83 bp
- **Upper Marker**: 276 bp, 251 bp, 91 bp
Microsatellite instabilities present in 10-15% of colon and gastric carcinomas

Study: 40 cases of colon carcinoma

5 microsatellite loci investigated

Results compared with traditional PAGE:

95% concordance rate
RNA Applications

- RNA QA/QC for Microarrays
- Gene Expression
- RNA QA/QC for qPCR
- RNA QA/QC for mPCR
- smallRNA QA/QC

[Images of RNA analysis graphs]

- Intact RNA: RIN 10
- Partially degraded RNA: RIN 5
- Strongly Degraded RNA: RIN 3
Agilent 2100 bioanalyzer: the industry standard in RNA QC

Electrophoretic sizing, quantitation and QC of XNA and Proteins on a small glass Chip as done traditionally on slab gels (Agarose or SDS-PAGE)

First commercially available Lab-on-a-Chip product (since October 1999)

Analysis of totalRNA, mRNA and small RNA samples in ng and pg concentration range

Standardized RNA integrity assessment with RIN* algorithm

Multi-analysis capabilities: DNA, RNA, Proteins and Flow Cytometry

\[ \text{RIN} = \text{RNA Integrity Number}, \text{ an Agilent patented algorithm to determine RNA quality in a normalized way} \]

January 2008: ~ 7200 citations
# RNA Kit Specifications

## Analytical Specifications

<table>
<thead>
<tr>
<th></th>
<th>RNA 6000 Nano total RNA</th>
<th>RNA 6000 Nano mRNA</th>
<th>RNA 6000 Pico total RNA</th>
<th>RNA 6000 Pico mRNA</th>
<th>Small RNA Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantitative range</strong></td>
<td>25–500 ng/μL</td>
<td>25–250 ng/μL</td>
<td></td>
<td></td>
<td>50–2000 pg/μL of purified mRNA in water</td>
</tr>
<tr>
<td><strong>Qualitative range</strong></td>
<td>5–500 ng/μL</td>
<td>25–250 ng/μL</td>
<td>50–5000 pg/μL in water</td>
<td>250–5000 pg/μL in water</td>
<td>50–2000 pg/μL of purified mRNA in water</td>
</tr>
<tr>
<td><strong>Sensitivity (S/N&gt;3)</strong></td>
<td>5 ng/μL in water</td>
<td>25 ng/μL in water</td>
<td>50 pg/μL in water 200 pg/μL in TE</td>
<td>250 pg/μL in water 500 pg/μL in TE</td>
<td>50 pg/μL in water**</td>
</tr>
<tr>
<td><strong>Quantitation reproducibility</strong></td>
<td>10% CV (within a chip)</td>
<td>10% CV (within a chip)</td>
<td>20 % CV (within a chip)</td>
<td>20 % CV (within a chip)</td>
<td>25 % CV (within a chip)</td>
</tr>
<tr>
<td><strong>Quantitation accuracy</strong></td>
<td>20 % CV*</td>
<td>20 % CV*</td>
<td>30 % CV*</td>
<td>30 % CV*</td>
<td></td>
</tr>
<tr>
<td><strong>Maximum sample buffer strength</strong></td>
<td>100 mM Tris, 0.1 mM EDTA or 125 mM NaCl or 15 mM MgCl₂</td>
<td>100 mM Tris, 0.1 mM EDTA or 125 mM NaCl or 15 mM MgCl₂</td>
<td>50 mM Tris, 0.1 mM EDTA or 50 mM NaCl or 15 mM MgCl₂</td>
<td>50 mM Tris, 0.1 mM EDTA or 50 mM NaCl or 15 mM MgCl₂</td>
<td>10 mM Tris, 0.1 mM EDTA</td>
</tr>
</tbody>
</table>

## Physical Specifications

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Analysis time</strong></td>
<td>30 minutes</td>
<td>30 minutes</td>
<td>30 minutes</td>
<td>30 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td><strong>Samples per chip</strong></td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td><strong>Sample volume</strong></td>
<td>1 μL</td>
<td>1 μL</td>
<td>1 μL</td>
<td>1 μL</td>
<td>1 μL</td>
</tr>
<tr>
<td><strong>Kit stability</strong></td>
<td>24 months at 4 °C</td>
<td>24 months at 4 °C</td>
<td>24 months at 4 °C</td>
<td>24 months at 4 °C</td>
<td>24 months at 4 °C</td>
</tr>
<tr>
<td><strong>25 chips per kit</strong></td>
<td>RNA Nano 12/chip = 300 samples/kit</td>
<td>RNA Pico 11/chip = 275 samples/kit</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined analyzing the RNA ladder as sample

** Measured for the 40 nt fragment of the Small RNA ladder
Features of the RNA 6000 Assays

total RNA
determine integrity and quality of total RNA
determination of RNA concentration
identify ribosomal peaks
calculate the ratio of ribosomal peaks (18S/28S or 16S/23S)

RNA integrity number (RIN)

mRNA
determine integrity and quality of mRNA samples
Determination of mRNA concentration
calculate % ribosomal RNA in mRNA samples
Problem Description

The ratio of ribosomal bands is not sufficient to describe RNA integrity!

RNA degradation is a gradual process.

Results have to be interpreted by visual inspection.

Overlay of electropherograms only works well for samples with the same concentration.

Instrument dependency in signal height.
RNA Quality Control: Assessing Total RNA Integrity

Typical first QC step after RNA sample prep prior to microarrays or real-time PCR

High quality total RNA

Partially degraded total RNA
Gel Chip Comparison

False Negative

False Positive
RNA QC in Routine Gene Expression Workflow

Cells / Culture

RNA isolation

Total RNA

RNA QC via Agilent 2100 bioanalyzer

RIN

RIN above threshold

Continue with downstream Experiment (Microarray, real-time PCR, etc.)
cRNA Hybridization - Workflow

Total RNA QC

mRNA QC

Cy3/ Cy5 Labeling

Array experiment

Data evaluation

cRNA fragmentation

Cy5-1 40 min
Cy5-1 30 min
Cy5-1 20 min
Cy5-1 10 min
Cy5-1 5 min

Agilent Technologies
Labeled cRNA Quality Check by NanoDrop

### Yield of cRNA

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Partial Degradation</th>
<th>Complete Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy3 Yield (ug)</td>
<td>9.2</td>
<td>7.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Cy5 Yield (ug)</td>
<td>8.5</td>
<td>7.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

### Incorporation of Cy Dye

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Partial Degradation</th>
<th>Complete Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy3 Incorporation (pmol/ug)</td>
<td>11.3</td>
<td>8.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Cy5 Incorporation (pmol/ug)</td>
<td>12.7</td>
<td>10.0</td>
<td>4.1</td>
</tr>
</tbody>
</table>
Labeled cRNA Quality Check by BioAnalyzer

**Cy3 labeled cRNA**

- Ladder
- Intact (RIN = 7.3)
- Partial Degradation (RIN = 5.2)
- Complete Degradation (RIN = 2.4)

**Cy5 labeled cRNA**

- Peak Size: 0.3K (Partial Deg.)
- Peak Size: 0.6K (Intact)
- Peak Size: 0.1K (Complete Deg.)

![Agilent Technologies logo]
Scatter Plots: Self vs. Self

- Intact (RIN = 7.3)
- Partial Degradation (RIN = 5.2)
- Complete Degradation (RIN = 2.4)
Scatter Plots: Intact vs. Degradation

- Intact (RIN = 7.3)
- Partial Degradation (RIN = 5.2)
- Complete Degradation (RIN = 2.4)

Intact vs. Partial Degradation

Intact vs. Complete Degradation
Conclusions

- Labeled cRNA from different levels of RNA degradation results in low Cy Dye incorporation and low yield of cRNA
- RNA integrity levels of starting material had serious impact on downstream gene expression microarray results
- The RIN is an effective tool that can be used to evaluate RNA integrity objectively
Ribosomal RNA contamination in mRNA samples

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>rRNA Contamination</th>
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</thead>
<tbody>
<tr>
<td>RNA 6000 ladder</td>
<td>-</td>
</tr>
<tr>
<td>Rat brain mRNA</td>
<td>-</td>
</tr>
<tr>
<td>Bovine kidney mRNA</td>
<td>-</td>
</tr>
<tr>
<td>Mouse testis mRNA</td>
<td>-</td>
</tr>
<tr>
<td>Mouse liver mRNA</td>
<td>-</td>
</tr>
<tr>
<td>Mouse kidney I mRNA</td>
<td>-</td>
</tr>
<tr>
<td>Mouse kidney II mRNA</td>
<td>-</td>
</tr>
</tbody>
</table>

**Mouse kidney I mRNA**

**Mouse kidney II mRNA**

**rRNA contamination: 19.5%**

**rRNA contamination: 1.7%**
Laser Microdissection – PALM MicroBeam System and RNA Pico kit

Laser microdissection

Laser pressure catapulting: Section after catapulting of selected area

Catapulted area in the collection device

Laser Microdissection and Pressure Catapulting (LMPC)

RNA extraction

RNA sample QC using the Agilent 2100 bioanalyzer and the RNA 6000 Pico LabChip kit
Analysis of Small RNA (using RNA 6000 Assay)

Small RNA fraction: < 200 nts  
e.g. miRNA, siRNA, snRNA, tRNA, 5S RNA  

Bioanalyzer allows discrimination of different profiles
New Small RNA Assay versus existing RNA Assay

RNA 6000Nano
Size range: 25-6000nt
Results: Integrity, Total RNA amount, gDNA contamination

NEW! Small RNA
Size range: 6-150nt
Results: miRNA amount, Ratio and amount of other Small RNA
Applications

The new small RNA Assay as a tool for:

Verification, comparison and optimization in the small RNA region:

- High sensitivity to detect low abundant fragments
- High resolution for ss oligos, miRNA, pre-, t-, 5S-RNA’s
- Compatible with Total RNA samples or purified small RNAs.
- Semi-quantitative for single stranded RNA.
- Semi- Denaturing
- Analysis up to 150nt

Plus: Qualitative assessment of dsDNA, siRNA or other hairpin RNA up to 150bp

(size separation and relative amount estimation)
Small RNA Assay specifications

**Analytical Range** 6 -150 nt (to avoid overlap)

**Sensitivity** 50 pg/µl 
(diluted Ladder - 40 nt fragment; S/N > 3:1)

**Quantitative range** 50 pg/µl – 2000 pg/µl 
(purified miRNA in water after extraction ~<200nt)

**Quantitation Reproducibility** 25 % CV 
(defined on Ladder)

**Max amount total RNA** 100 ng/µl total RNA

**Carryover** Below detection limit
Protein Applications

Protein Purification
Protein Expression
Protein Production
Food Analysis
Purity and QA/QC

Silver stained PA-gel
2100 Pseudo gel view

\[ \gamma = 12.791 \times 0.8578 \]
\[ R^2 = 0.9964 \]
## Bioanalyzer Protein Kit portfolio

<table>
<thead>
<tr>
<th>Kit Name</th>
<th>Prod Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent Protein 80 kit</td>
<td>5067-1515</td>
</tr>
<tr>
<td>Agilent Protein 230 kit</td>
<td>5067-1517</td>
</tr>
<tr>
<td>Agilent High Sensitivity Protein 250 kit</td>
<td>5067-1575</td>
</tr>
</tbody>
</table>

### P 80
- **Range:** 5 - 80 kDa
- **Sensitivity:** Coomassie
- **Samples:** 10
- **Samples**:
  - Antibodies (reduced)
  - Small Proteins

### P 230
- **Range:** 14 - 230 kDa
- **Sensitivity:** Coomassie
- **Samples:** 10
- **Samples**:
  - Antibodies (all types)
  - Standard Proteins

### HSP 250
- **Range:** 10 - 250 kDa
- **Sensitivity:** 1 pg/µL BSA on Chip
- **Samples #:** 10 per Chip
- **Chips #:** 10 per Kit
- **Labeling Conc.:** 1 ng – 1 µg/µL

### Coomassie Range (5 ng/µL BSA)

### Silver stain Range (200 pg/µL BSA)
Protein Kit Specifications

<table>
<thead>
<tr>
<th>Product No. 5067-1515</th>
<th>Product No. 5067-1517</th>
<th>Product No. 5067-1575</th>
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</thead>
<tbody>
<tr>
<td><strong>Analytical specifications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sizing range</td>
<td>5-80 kDa</td>
<td>14-230 kDa</td>
</tr>
<tr>
<td>Typical sizing resolution</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Typical sizing accuracy</td>
<td>10% CV (CAII, BLG)</td>
<td>10% CV (BSA, CAII)</td>
</tr>
<tr>
<td>Sizing reproducibility</td>
<td>3% CV (CAII, BLG)</td>
<td>3% CV (BSA, CAII)</td>
</tr>
<tr>
<td>Sensitivity (Signal/Noise &gt; 3)</td>
<td>6 ng/μL CAII (15 ng/μL BSA) in PBS, 10 ng/μL (CAII) in 0.5 M NaCl (30 ng/μL BSA in 0.5 M NaCl)</td>
<td>6 ng/μL CAII (15 ng/μL BSA) in PBS 30 ng/μL (BSA) in 0.5 M NaCl</td>
</tr>
<tr>
<td>Quantitative range</td>
<td>60-2000 ng/μL CAII in PBS</td>
<td>15-2000 ng/μL CAII, 30-2000 ng/μL BSA in PBS</td>
</tr>
<tr>
<td>Qualitative range</td>
<td>6-4000 ng/μL CAII and BLG</td>
<td>6-5000 ng/μL CAII, 15-5000 ng/μL BSA in PBS</td>
</tr>
<tr>
<td>Quantitation reproducibility</td>
<td>20% CV (CAII, BLG)</td>
<td>20% CV (BSA, CAII)</td>
</tr>
<tr>
<td><strong>Physical specifications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis run time</td>
<td>30 minutes</td>
<td>25 minutes</td>
</tr>
<tr>
<td>Number of samples</td>
<td>10 samples/chip</td>
<td>10 samples/chip</td>
</tr>
<tr>
<td>Sample volume</td>
<td>4 μL</td>
<td>4 μL</td>
</tr>
<tr>
<td>Kit stability</td>
<td>4 months (for storage temperature see individual box)</td>
<td>4 months (for storage temperature see individual box)</td>
</tr>
<tr>
<td>Compatible buffers</td>
<td>List of compatible buffers</td>
<td>List of compatible buffers</td>
</tr>
</tbody>
</table>

* Prior to measurement on Chip we recommend within the High Sensitivity Protein 250 labeling protocol to dilute the labeled sample by a factor of 200.
Staining, Destaining and Detection (P-80 and P-230)

SDS + dye

protein

micelles

Avoiding high background, providing better sensitivity

If no dilution was done the micelles would result in high background and low sensitivity

destain

detection

low background good signal to noise ratio SDS conc. below CMC
Clone Selection based on Protein Expression

Example measured with Protein 50 kit
Monitoring of Protein Purification Process

Example measured with Protein 200 plus kit

GFP Fusion Protein Analysis

2100 bioanalyzer: gel-like image
2100 bioanalyzer: electropherogram

Courtesy of P. Sebastian and S.R. Schmidt
GPC-Biotech AG, Martinsried, Germany
Expression of a Recombinant Protein in *E. coli*

- Optimization of Fermentation and Induction Conditions

**Fluorescence vs Time (seconds)**

- **soluble protein fraction**
- **recombinant protein**
- **solubilized inclusion bodies**
  (50 mM Tris pH 7.5, 100 mM DTT, 8M urea)
Quality control of the Depletion of High Abundance Proteins in Human Serum

Depletion of High Abundance Proteins by Agilent MARS HPLC columns

Fractions checked by 2100
Quality Control of Antibodies

Determine the half antibody content in IgG preparations

Antibody analysis under reducing and non-reducing conditions

Absolute Quantitation of IgG samples

Antibody

- 90 kDa

Intact antibody

16% half antibody

90 kDa

160 kDa

Ab reduced

Ab non-reduced

light chain

heavy chain

light + heavy chain

intact antibody
Analysis of Antibody Stability – stress test

A

B

Antibody 1 - Standard

Antibody 1 - 1 month 40 °C

Antibody 2 - Standard

Antibody 2 - 12 weeks 40 °C
Combination of IEF with SDS-PAGE
Agilent 3100 OFFGel Fractionator + 2100 bioanalyzer
Description of the new HSP-250 Assay (Direct labeling reaction, silver stain sensitivity)

- Reach and beat traditional „silver stain sensitivity“
- Offer solid quantitation for a large dynamic range

**Target Applications:**

- Protein QA/QC  
  reliable quantitation of main compound besides minor impurities
- Protein detection at lowest concentrations in research

**High Sensitivity Protein 250 Kit 5067-1575 content is:**

- 10 Chips (100 samples)
- Labeling Kit (Dye and Reagents)
- 2100 Separation Kit (Gel, Marker, Ladder, Buffer)
- User Documentation (Quick Start Guide & Labeling Protocol)
Extended experimental workflow

Transfer to suitable buffer
(precipitation, ultrafiltration, buffer exchange spin columns)

5-90 min

Labeling with dye
(N-hydroxy-succinimidyl ester chemistry)

40 min

Sample preparation for 2100
(SDS denaturation, dilution if desired)

5 min

Analysis on 2100

35 min

Data
Principle of High Sensitivity 250 Protein Staining

Sample protein

chemically activated fluorescent dye

NHS

pH 8.5
0°C

Covalently labeled

Ethanolamine

NH₄EOH

30 min
10 min

Step 1
Labeling Reaction

Step 2
SDS-Denaturation

SDS + 95°C

5 min

labeled Protein

Step 3
Separation on Chip

Separation & Detection

Laser induced Fluorescence

30 min

Agilent Technologies

Page 70
Reproducibility of Labeling Reaction: Ladder

Rugged Labeling reaction:
Reproducible reaction provides comparable signal intensities. Homogenous labeling without extra band broadening.

No deviation in peak width is indicating a constant number of dye per protein molecule and proves a stable protocol.
Sensitivity: Silver Staining vs. Bioanalyzer

**Highest sensitivity:**
Labeled proteins can be measured down to pg/µL concentrations loaded on Chip

Direct comparison of samples run on SDS-PAGE with Silver staining and on 2100 Bioanalyzer.

Concentrations are given per lane (as total concentration of 7 different proteins)
Linear Dynamic Range Test: IgG

Linear dynamic range:
Quantification of labeled IgG from 10 pg/µL to 100 ng/µL
averages ± SD of 7 measurements (7 chips, 1 chip lots, 4 instruments)

4 orders of linear dynamic range allows to quantify an 0.05% impurity besides the main peak in a single run
2100 Bioanalyzer Compliance

2100 expert software
- One version for all assays
- Declaration of system validation

2100 expert security pack
- 21 CFR part 11 compliance
- Electronic records
- Electronic signatures
- Audit trails

2100 bioanalyzer
- IQ and OQ/PV services
- Declaration of conformity

Chips and reagents
- Declaration of conformity
# 2100 kits for Protein applications

## Protein Kits – Coomassie stain sensitivity

<table>
<thead>
<tr>
<th>Number</th>
<th>Kit</th>
<th>Max # of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-1517</td>
<td>Agilent Protein 230 Kit</td>
<td>250</td>
</tr>
<tr>
<td>5067-1518</td>
<td>Agilent Protein 230 Reagents</td>
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</tr>
<tr>
<td>5067-1515</td>
<td><strong>Agilent Protein 80 Kit</strong></td>
<td>250</td>
</tr>
<tr>
<td>5067-1516</td>
<td>Agilent Protein 80 Reagents</td>
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</table>

## Protein Kits – Silver stain Sensitivity

<table>
<thead>
<tr>
<th>Number</th>
<th>Kit</th>
<th>Max # of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-1575</td>
<td><strong>High sensitivity Protein 250 Kit</strong></td>
<td>100</td>
</tr>
<tr>
<td>5067-1576</td>
<td>High sensitivity Protein 250 Reagents</td>
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</tr>
<tr>
<td>5067-1577</td>
<td>High sensitivity Protein 250 Labeling Reagents</td>
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</tr>
<tr>
<td>5067-1578</td>
<td>High sensitivity Protein 250 Ladder</td>
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</tr>
</tbody>
</table>
### 2100 kits for Cell Assay and DNA applications

#### Cell Fluorescence Kits

<table>
<thead>
<tr>
<th>Number</th>
<th>Kit</th>
<th>Max # of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-1519</td>
<td>Agilent Cell Kit</td>
<td>150</td>
</tr>
<tr>
<td>5067-1520</td>
<td>Cell Checkout Kit</td>
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</tbody>
</table>

#### DNA Kits

<table>
<thead>
<tr>
<th>Number</th>
<th>Kit</th>
<th>Max # of samples</th>
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</thead>
<tbody>
<tr>
<td>5067-1504</td>
<td>Agilent DNA 1000 Kit</td>
<td>300</td>
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<tr>
<td>5067-1505</td>
<td>Agilent DNA 1000 Reagents</td>
<td></td>
</tr>
<tr>
<td>5067-1506</td>
<td>Agilent DNA 7500 Kit</td>
<td>300</td>
</tr>
<tr>
<td>5067-1507</td>
<td>Agilent DNA 7500 Reagents</td>
<td></td>
</tr>
<tr>
<td>5067-1508</td>
<td>Agilent DNA 12000 Kit</td>
<td>300</td>
</tr>
<tr>
<td>5067-1509</td>
<td>Agilent DNA 12000 Reagents</td>
<td></td>
</tr>
</tbody>
</table>
# 2100 kits for RNA applications

## RNA Kits

<table>
<thead>
<tr>
<th>Number</th>
<th>Kit</th>
<th>Max # of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>5067-1511</td>
<td>Agilent RNA 6000 Nano Kit</td>
<td>300</td>
</tr>
<tr>
<td>5067-1512</td>
<td>Agilent RNA 6000 Nano Reagents</td>
<td></td>
</tr>
<tr>
<td>5067-1529</td>
<td>Agilent RNA 6000 Nano Ladder</td>
<td></td>
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<tr>
<td><strong>5067-1513</strong></td>
<td><strong>Agilent RNA 6000 Pico Kit</strong></td>
<td><strong>275</strong></td>
</tr>
<tr>
<td>5067-1514</td>
<td>Agilent RNA 6000 Nano Reagents</td>
<td></td>
</tr>
<tr>
<td>5067-1535</td>
<td>Agilent RNA 6000 Nano Ladder</td>
<td></td>
</tr>
<tr>
<td><strong>5067-1548</strong></td>
<td><strong>Agilent Small RNA Kit</strong></td>
<td><strong>275</strong></td>
</tr>
<tr>
<td>5067-1549</td>
<td>Agilent Small RNA Reagents</td>
<td></td>
</tr>
<tr>
<td>5067-1550</td>
<td>Agilent Small RNA Ladder</td>
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</tbody>
</table>
**Determination of PCR Product Impurity**

### Quantitative data from Agilent 2100 bioanalyzer

<table>
<thead>
<tr>
<th>Sample</th>
<th>c (DNA)</th>
<th>main peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 bp PCR</td>
<td>41.4 ng/ul</td>
<td>40.7 ng/ul</td>
</tr>
<tr>
<td>300 bp PCR 1:4</td>
<td>9.6 ng/ul</td>
<td>9.6 ng/ul</td>
</tr>
</tbody>
</table>

**300 bp**

Impurity level: < 2%

**3000 bp**

Impurity level: > 50%

<table>
<thead>
<tr>
<th>Sample</th>
<th>c (DNA)</th>
<th>main peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000 bp PCR</td>
<td>61.9 ng/ul</td>
<td>40.7 ng/ul</td>
</tr>
<tr>
<td>3000 bp PCR 1:4</td>
<td>14.8 ng/ul</td>
<td>9.8 ng/ul</td>
</tr>
</tbody>
</table>