Amersham ImageQuant™ 500 biomolecular imager

IMAGING SYSTEMS, SOFTWARE, AND ACCESSORIES

Amersham ImageQuant™ 500 imaging system is a cooled CCD imager for imaging of chemiluminescent Western blots, fluorescent protein and DNA gel stains, and white light imaging of colorimetric stains and markers (Fig 1).

ImageQuant™ 500 imaging system offers:

- Sensitive detection at picogram sample levels
- Automatic overlay function for imaging of chemiluminescent samples and colored molecular weight markers
- Rapid cooling to –25°C in less than five minutes at room temperature for quick start up
- Easy system installation in a few minutes without a service engineer
- Compact integrated design with a small footprint
- Flexible storage of image on a USB memory stick; on the system; or in a network connection folder

Fig 1. ImageQuant™ 500 imaging system is a fast, easy-to-use system for chemiluminescence, colorimetric, and fluorescence image capture.

Description

ImageQuant™ 500 imaging system consists of a touchscreen, sample chamber, USB port, camera, and light sources (Fig 2). The 16-bit, 8.3 megapixel CCD camera is fitted with a 30 mm (F1.4) fixed focus lens. The CCD camera is ready for use in minutes, and advanced Peltier cooling and binning significantly reduce noise levels, increasing both the sensitivity and linearity of the system.

The camera is designed for chemiluminecense detection with regard to sensitivity and dynamic range. In addition, the system has three LED light sources; a combined blue epi-/UV epi-light and a white epi-light. The combined blue epi-/UV epi-light allows imaging of fluorescence signals above 560 nm. This feature makes the camera well suited for DNA stain imaging with SYBR™ Green and ethidium bromide (EtBr) as well as with common total protein stain dyes.

The white epi-light source (470 to 635 nm) is for documentation of colorimetric markers and stains, such as Coomassie™ Blue or silver. Moreover, the white light mode can be combined with the chemiluminescent mode to generate an overlay image between a colored marker and chemiluminescent sample. The overlay function provides a fast and easy way to verify the molecular weight of detected bands and identification of band of interest if multiple bands are generated. Signal intensity can be detected automatically or exposure time can be set manually by the operator.

ImageQuant™ 500 imaging system is a practical and affordable solution to individual researchers or laboratories that perform routine imaging or need extra imaging capacity to supplement existing imaging equipment or darkroom use. The 30 × 28 cm footprint has the same area as a typical laptop computer, which allows the imager to fit neatly on almost any lab bench without the need to connect ancillary equipment.

1 Touchscreen user interface
2 Fluorescence filter
3 Power switch
4 Sample tray
5 Cabinet door
6 USB port

Fig 2. ImageQuant™ 500 imaging system with its intuitive software is easy to install and ready for capturing of images in less than five minutes.

Simple workflow

A typical workflow is fast and simple (Fig 3). After power-up, it takes less than five minutes for the system to cool to an operating temperature of –25°C. Insert the sample tray with sample into the system and select the imaging and exposure modes from the screen. After image capture, the signal intensity for the band of interest can be displayed directly on the touchscreen. If you have a particular band of interest, you can use the semiauto exposures mode to capture an optimized image of the band of your interest.

Fig 3. ImageQuant™ 500 imaging system takes minutes to install, is compact, and can capture images in five minutes.

In case you wish to try several exposure conditions, the software offers an incremental exposure mode in which you will have images with a series of different exposure times. After, you can pick up the image you prefer. The exposure time can be adjusted manually by the operator for additional images if fine-tuning of the results is desired. Images can be saved to a USB memory stick, on the system under the ***Library*** tab (Fig 4), or transferred to a folder via a network connection.

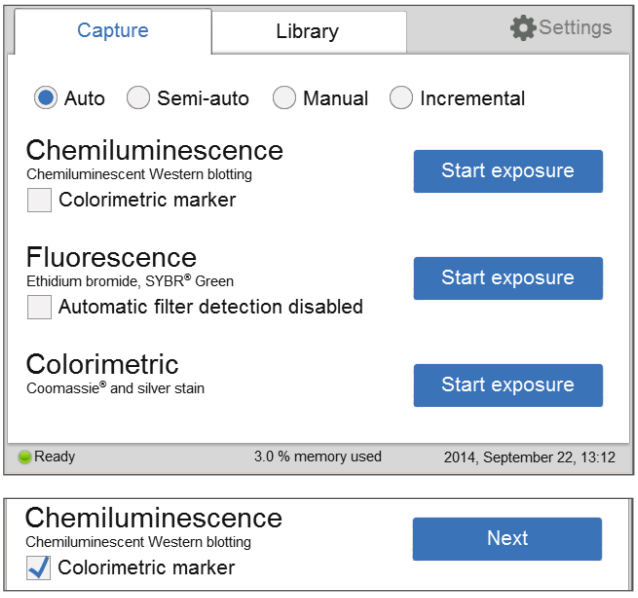

Fig 4. The main menu displays the capture methods and has automatic exposure (***Auto***) as default. If the sample has a colorimetric marker, tick the ***Colorimetric marker*** check box.

User-friendly image capture software

ImageQuant™ 500 imaging system operation is fully automated. There is no need for focusing, insertion of light sources or other adjustments or calibrations after starting the system. The system is operated from a touchscreen with clearly defined buttons and tabs. The main menu displays the capture modes (Fig 4). ImageQuant™ 500 imaging system uses automatic exposure as default when the system is started. Automatic exposure uses a short pre-exposure of the sample to determine the signal intensity. Based on this information, the system chooses an exposure time for optimized signal without saturating the image, allowing accurate quantification of the sample.

If a chemiluminescent sample has a colorimetric marker, tick the ***Colorimetric marker*** check box (Fig 4). When the box is ticked, three image files are created: a chemiluminescence 16-bit .tif image (of the sample), a white light 16-bit .tif image (of the marker), and a combined color .jpg image of both the sample and the marker.

In semiauto image capture mode, an automated exposure is made based on an area of interest defined by the user. With incremental exposure mode, images with a series of different exposure times are captured. Exposure times are also easy to set manually.

During exposure, a progress screen indicates the image capture progress. When the capture is complete, an image is displayed on the touchscreen. If desired, tools such as ***Contrast, Intensity, Zoom,*** and ***Invert display*** are readily available to examine the image in detail (Fig 5). The intensities can be verified directly on the touchscreen and exposure time can be adjusted without external analysis software. If additional analysis is desired, the images can be transferred to a PC for analysis with ImageQuant™ TL 10.1 (sold separately).

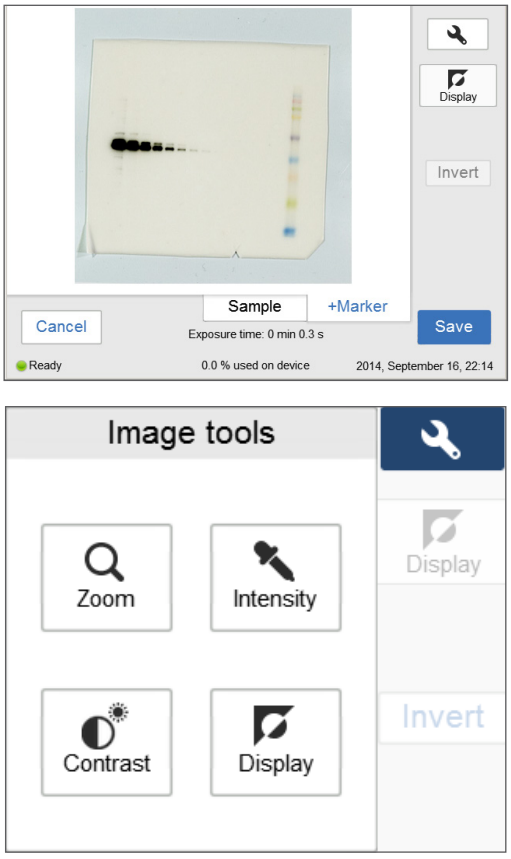

Fig 5. An example of chemiluminescent image with colorimetric marker. The call out shows the image enhancement tools that can be used to examine the image in detail.

Imaging applications

The following applications illustrate the performance and flexibility of ImageQuant™ 500 imaging system in everyday use.

Chemiluminescent Western blotting detection of transferrin in the picogram range

Quantitative Western blotting requires a signal response proportional to the amount of protein. A broad dynamic range, that is, a linear signal response over a wide range of protein amounts, allows users to simultaneously study both high and low abundant proteins quantitatively (Fig 6). Amersham ECL™ Prime and Amersham ECL™ Select reach a limit of detection in the picogram range with ImageQuant™ and a dynamic range covering three orders of magnitude.

Sample:	Two-fold dilution of transferrin from 5 ng to 2.4 pg
Membrane:	Amersham Hybond™-P
Blocking:	Amersham ECL™ Prime Blocking Agent in 2% PBS-T
Primary antibody:	Rabbit anti-transferrin (1:3000)
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1:30 000)
Detection:	Amersham ECL™ Prime
Imaging method:	Chemiluminescence
Limit of detection (LOD):	4.9 pg
Dynamic range (DR):	3.0 orders of magnitude
Analysis:	ImageQuant™ TL software

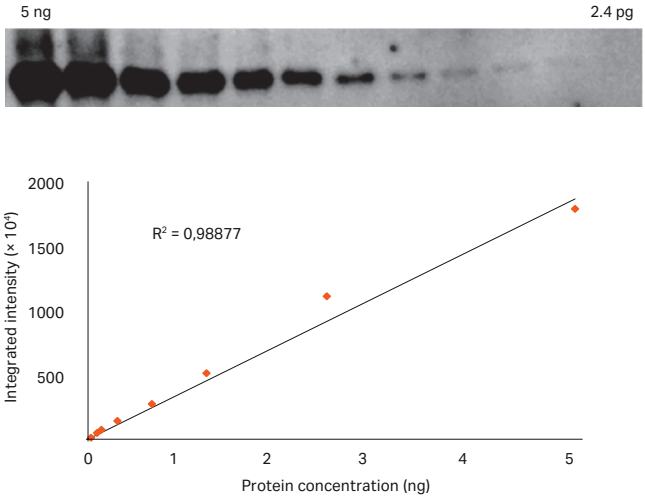

Fig 6. Evaluation of dynamic range and limit of detection. Detection of chemiluminescent signals with ImageQuant™ 500 imaging system in a two-fold transferrin dilution series. The data was analyzed in ImageQuant™ TL. The result demonstrates a dynamic range of three orders of magnitude and a limit of detection of 4.9 pg transferrin.

Illustration of automatic overlay functionality

ImageQuant™ 500 imaging system offers chemiluminescence imaging with an automatic overlay function, a feature that sets a new standard for imaging. The function allows simultaneous imaging of a chemiluminescence sample and a colored molecular weight marker (Fig 7). The overlay picture presents the marker in color and will be helpful to estimate the size of the detected proteins and to orient the membrane.

Sample:	Two-fold dilution of HeLa cell lysate starting at 10 µg
Marker:	Full-Range Rainbow™ Molecular Weight Marker
Membrane:	Amersham Hybond™-P
Blocking:	Amersham ECL™ Prime Blocking 3% in PBS-T
Primary antibody:	Rabbit anti-ERK 1/2 (1:5000)
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1:30 000)
Detection:	Amersham ECL™ Prime
Imaging:	Amersham ImageQuant™ 500 imaging system
Imaging method:	Chemiluminescence + colorimetric marker
Analysis:	Amersham ImageQuant™ TL software

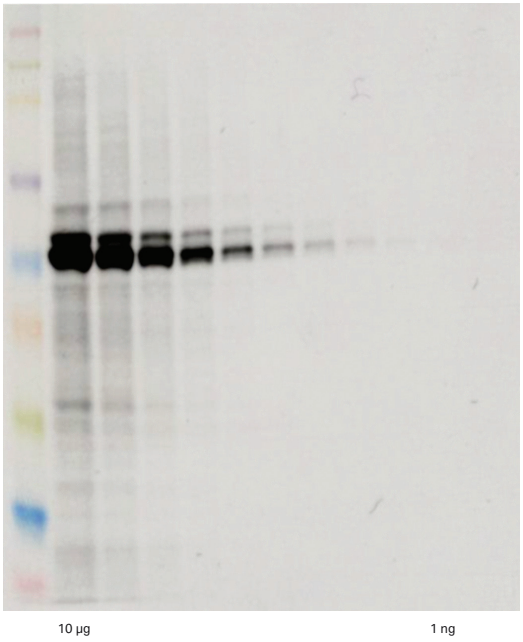

Fig 7. The chemiluminescence mode allows simultaneous imaging of chemiluminescent signals and a colored molecular weight marker. The overlay functionality provides a fast and easy way to verify the molecular weight of detected bands, and identification of band of interest if multiple bands are generated. Amersham ECL™ Prime detection of ERK1/2 in cell lysates from HeLa cells with ImageQuant™ 500.

Dynamic range provided by ImageQuant™ 500 imaging system compared with X-ray film

With low detection limits, ImageQuant™ 500 imaging system combined with Amersham ECL™ Prime or Amersham ECL™ Select provides high quality data in applications that demand high sensitivity over a wide dynamic range. Figure 8 illustrates the advantage of CCD camera imaging compared with X-ray film when working with quantitative applications. ImageQuant™ 500 imaging system gives a linear correlation between protein quantity and signal intensity of three orders of magnitude. Detection with X-ray film results in saturation of the five most intense bands and a linear correlation between protein and signal intensity of 1.2 orders of magnitude.

Sample:	Two-fold dilution of HeLa cell lysate starting at 5 µg
Membrane:	Amersham Hybond™-P
Blocking:	Amersham ECL™ Prime Blocking 2% in PBS-T
Primary antibody:	Rabbit anti-ERK 1/2 (1:10 000)
Secondary antibody:	HRP-conjugated anti-rabbit IgG (1:100 000)
Detection:	Amersham ECL™ Select
Imaging:	Amersham ImageQuant™ 500 imaging system using chemiluminescence, 5 s exposure Amersham Hyperfilm™ ECL, ImageScanner III, 5 s exposure
Analysis:	Amersham ImageQuant™ TL v8.1 software

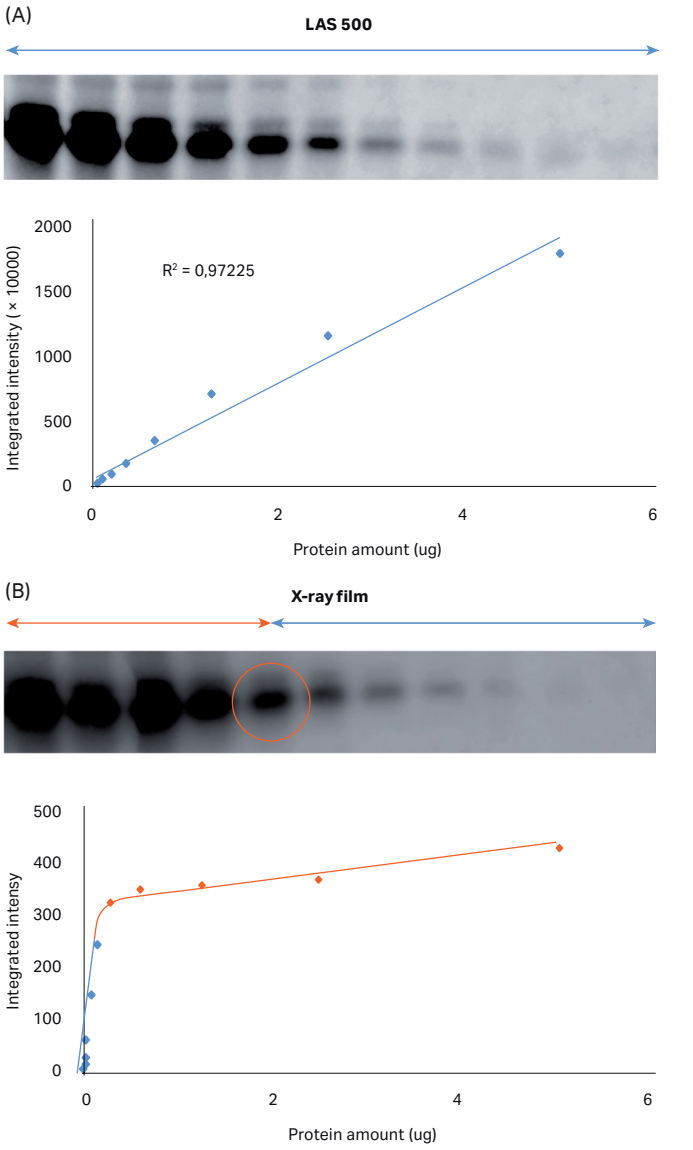

Fig 8. Comparison of dynamic range between ImageQuant™ 500 imaging system and X-ray film. Amersham ECL™ Select detection of ERK1/2 in cell lysates from HeLa cells (A) by chemiluminescence using ImageQuant™ 500 imaging system and by (B) Hyperfilm X-ray film. The correlation between signal and protein amount is indicated in the two diagrams. Protein above the points indicated in red could not be quantified using X-ray film.

Sensitive imaging of total protein stains

Proteins can be visualized by treating a gel with a total protein stain after performing PAGE. The most commonly used stains are Coomassie Blue or silver staining. Fluorescent staining methods such as Deep Purple™ Total Protein Stain has the advantage of being highly sensitive and gives a broad dynamic range in combination with ImageQuant™ 500 imaging system. Figure 9 illustrates a two-fold dilution series of HeLa cell lysate. The gels were stained with Coomassie Blue and Deep Purple and results were documented by ImageQuant™ 500.

Sample:	Two-fold dilution series of HeLa cell lysate from 10 µg to 625 ng
Detection:	Coomassie Blue (A) Deep Purple (B)
Imaging:	Amersham ImageQuant™ 500 imaging system
Imaging method:	Colorimetric (A) Fluorescence (B)
Analysis:	Amersham ImageQuant™ TL software

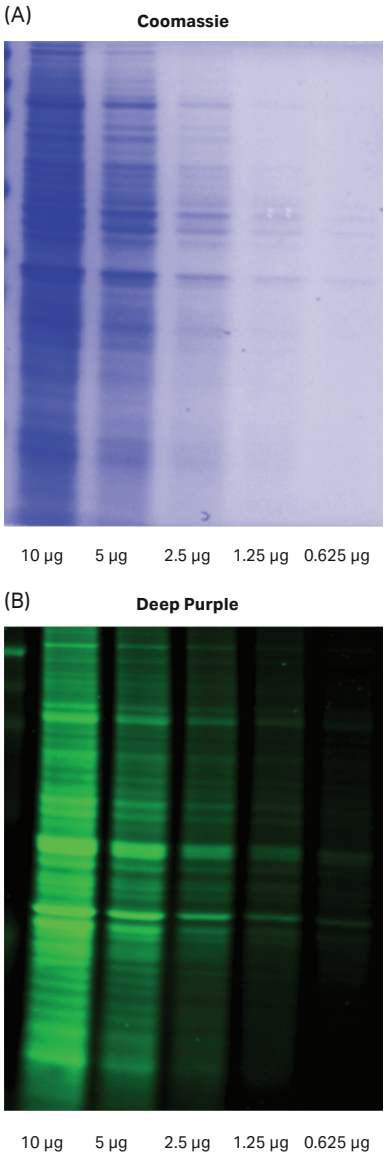

Fig 9. Protein stains after SDS-PAGE. (A) Colorimetric method with Coomassie Blue. (B) Fluorescent method with Deep Purple Total Protein Stain.

DNA stain imaging

Electrophoretic separation of DNA is a common technique that is typically used for analysis of vector cleavages, and verification of successful PCR. Traditionally, EtBr has been used for visualizing DNA, but the carcinogenic property of the compound has resulted in development of a number of alternative DNA stains. One safer alternative to EtBr is to use SYBR Green. Figure 10 shows staining of a pGEX vector with SYBR Green and MassRuler™ DNA ladder with EtBr.

Sample:	5 ng Kilobase DNA marker and 48 ng pGEX vector (4900 kb) (A) Two-fold dilution series of Massruler DNA Ladder from 2 µg down to 31.25 ng (B)
Gel:	0.8% agarose in TAE, 5 mm (A) 1% agarose in TBE, 5 mm (B)
Detection:	SYBR Green (A) EtBr (B)
Imaging:	Amersham ImageQuant™ 500 imaging system
Analysis:	Amersham ImageQuant™ TL software

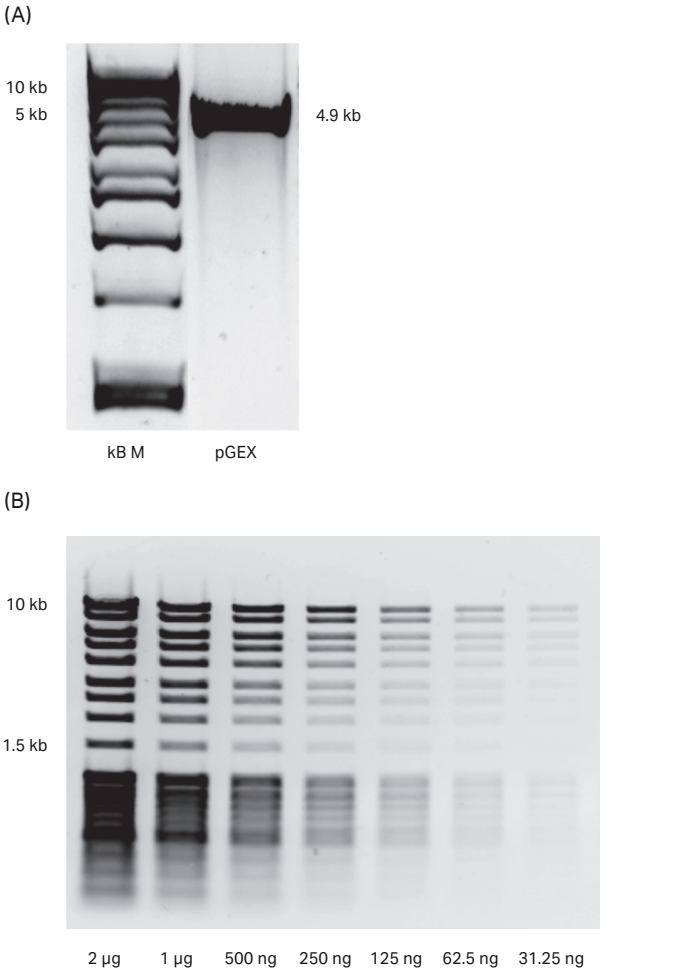

Fig 10. DNA samples stained with (A) SYBR Green and (B) EtBr. ImageQuant™ 500 imaging system was used for image capture and ImageQuant™ TL for analysis.

Technical features

Table 1. ImageQuant™ 500 imaging system specifications

Image sensor	Peltier cooled, 8.3 megapixel CCD
Image sensor temperature	-25°C
Cooling time	< 5 min
Lens	F1.4/30 mm
Light source	UV epi-light: 365 nm Blue epi-light: 460 nm White epi-light: 470 to 635 nm
Operation	Fully automated (auto exposure, no focus or other adjustment or calibrations are required)
Exposure modes	Auto, semiauto, manual, and incremental
Sample size (max.)	105 × 105 mm
Greyscale	65 536 levels (16-bit)
Exposure time	1/10 to 1 h*
Dynamic range (theoretical)	4.8 orders of magnitude
Image output	Grayscale 16-bit (.tiff) Color image (.jpg)
Interface	USB 2.0 Ethernet
Dimensions	300 (W) × 570 (H) × 280 (D) mm
Weight	15 kg
Input voltage	100 to 240 V
Line frequency	50/60 Hz
Max. power	200 W
Operating temperature	15°C to 28°C
Humidity conditions	15% to 70% (no condensation)

* With the increment mode, up to 12 h

Ordering Information

Product	Quantity	Code number
ImageQuant™ 500 imaging system	1	29655893

Accessories included	Quantity	Code number
Protein tray	1	29-0050-67
DNA tray	1	29-0050-66
White insert for gels or membranes with colorimetric markers and stains	1	29-0050-69
Orange filter (560LP) for detection of fluorescence above 560 nm	1	29-0050-68
Accessories case	1	29-0050-70

Software	Code number
IQTL 10.1 Node locked license	29705686
QTL 10.1 Floating license	29705685
IQTL 10.1 MAC OS node locked license	29705697

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CY12756-28Jan22-DF