



Features and Applications of the Jenway 62 series fluorimeters



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■ General features of the 62 series fluorimeters

The Jenway 62 series fluorimeter range consists of three models designed to cover a wide range of applications. The model 6280 is ideal for the most sensitive determinations with emission wavelengths up to 650nm. Where higher emission wavelengths are required, the model 6285 with its red-enhanced detector can extend the range up to 850nm. For less sensitive applications with a broader wavelength range, the model 6270 will meet the necessary requirements.

All models in the 62 series range enable simple raw fluorescence measurements to be made without the need to calibrate. These comparative fluorescence levels can be used as a basic indicator of the amount of fluorescent material present or for method evaluation procedures. If precise concentration measurements are required, these can be made by calibration with a blank and standard solution. Where expected sample concentrations may cover a wide range, a calibration curve can be created with up to six standards using a simple step-by-step procedure.



All three models offer intuitive operation with a user interface based on logical menus that can be navigated from the simple keypad. Up to 20 methods can be created without restriction and be saved for future use; they can also be locked against accidental change by password entry, ensuring data integrity. The permanent time and date tag on every stored reading further enhances Good Laboratory Practice, while calibration reminders and operator identity can also be entered to support conformance and traceability of operation.

With press-to-read operation and Total Energy Transfer (TET) technology, the output of the high-energy xenon lamp is maximised and its expected life extended so that it should never need replacing in normal use. The high quality optics are complemented by the Intelligent Filter Modules (IFM) that can be identified by the system; this enables error messages to be generated and displayed should the wrong filters be fitted or their positions be incorrect.

All models are powered from an external, universal mains adapter suitable for use from 90Vac to 264Vac, while the 12V dc input enables use of the fluorimeters in vehicles or from suitable battery packs. An optional carry case is available for protection during transportation.

Where temperature controlled fluorescence studies are necessary an optional electrically heated sample holder is easily fitted while for continuous flow analysis an external sipper pump and a wide range of flow through cuvettes are also available. Timed measurements can be made for enzyme kinetics studies or for the monitoring of changes in static or flowing samples. Results can be displayed as data or in graphical form. All models can also be used with the optional DataWay PC software to log data, construct graphs, transfer data to Excel® or other Windows programmes and save data to PC files.

In this brochure we highlight some of the features of the fluorimeters and provide details of applications in which they can be used. Application notes and protocols for specific methods can be downloaded from http://www.jenway.com/notes_Fluorimeters.asp.



■ Model 6280

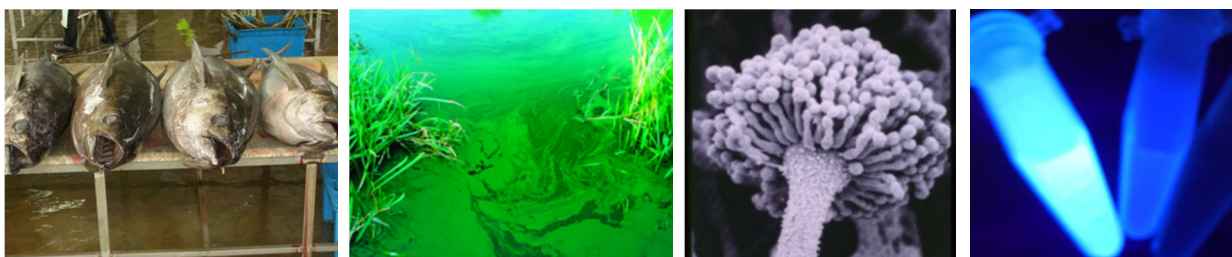
The model 6280 is recommended for the most sensitive determinations with emission wavelengths up to 650nm. The model 6280 uses a photomultiplier tube (PMT) detector, the sensitivity of which can be adjusted by altering the gain setting. This ensures that a wide dynamic range of sample concentrations can be detected with the highest sensitivity. The model 6280 is ideal for a broad range of applications in food and beverage analysis, including determination of histamine, quinine, aflatoxins and vitamins. It also has a wide range of uses in biochemistry and the life sciences for measurement of parameters such as DNA/RNA concentration, protein, steroids, enzymes and drugs. For water and environmental analysis it has applications in dye tracing (flow measurement), cyanobacteria identification and ammonium measurement amongst many others.

■ Model 6285

The model 6285 has a red-enhanced PMT detector making it ideal for sensitive applications which require detection at higher emission wavelengths up to 850nm. The measurement of chlorophyll *a*, algal pigments and Rhodamine B are three examples of where this enhanced detection wavelength range is required.

■ Model 6270

The model 6270 is a lower cost model that can be used for less sensitive applications with a broader wavelength range. It is ideal for applications where higher levels of fluorescence are encountered as well as for experiments investigating the principles of fluorimetry and similar projects in education and training. The rugged silicon photodiode detector makes the model 6270 ideal for field-based applications.

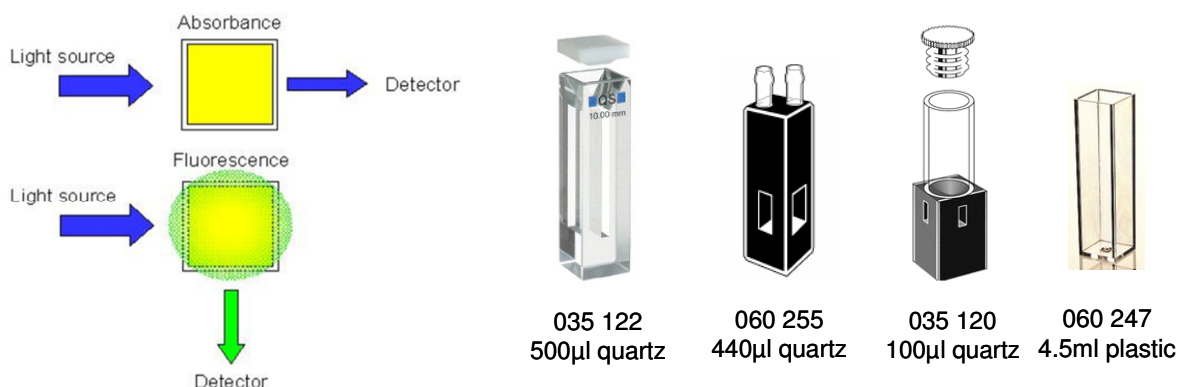


■ Accessories

To increase the flexibility of the Jenway fluorimeters, a number of accessories are available to optimise sample handling, maintain stable assay conditions and facilitate data collection and storage.

• Cuvettes

In a fluorimeter, the fluorescence of the sample is measured at 90° to the incident light otherwise the detector will see light from the lamp which is not due to fluorescence of the actual sample. Therefore, unlike spectrophotometer cuvettes, fluorimeter cuvettes have windows on adjacent walls.



A full range of cuvettes is available to accommodate samples of different volumes and wavelength ranges. These include quartz cells for work in the UV range as well as glass and disposable plastic cuvettes for work in the visible wavelengths. Jenway also offer a range of quartz and glass flow-through cuvettes.

- **Sipper Pump (632 001)**

For high throughput applications or where there is a continuous flow of samples such as fraction collecting, a sipper pump in combination with a flow cell can greatly reduce the amount of sample handling required. This programmable peristaltic pump can be set to dispense as little as 75µl of sample into the chamber of a suitable flow-through cuvette, with selectable air segmentation and rinse cycles to match varying sample requirements. Alternatively, in continuous mode it can pump a continuous stream of sample through the cuvette from a flowing or bulk sample.



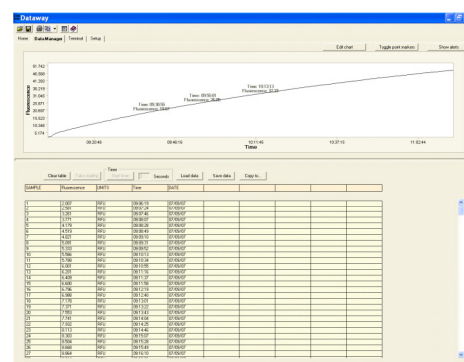
- **Heated cell system (628 200)**

Fluorescence intensity is highly dependent on temperature and fluctuating ambient conditions can affect results. In addition, many biological fluorimetric assays require temperature conditions above ambient levels and this can be achieved using the electrically heated sample holder. The sample holder is controlled by a separate unit which plugs directly into the back of the fluorimeter. With temperature regulation to $\pm 0.1^\circ\text{C}$, from 2°C above ambient to 60°C , it will fulfill the requirements of most applications.



- **DataWay (050 501)**

For kinetic assays or time course experiments where numerous and continuous readings are required, the 62 series fluorimeters can be used with DataWay data acquisition software. Connecting the instrument to the PC is very simple using the interface cables supplied with the software. DataWay can be set up to take readings at timed intervals, which can then be plotted in real time for an instant view of the experiment progress. All results are time and date stamped for GLP purposes.



The active graphing function allows the real time display of captured data; the co-ordinates of each data point can be displayed by moving the cursor along the graph. Multiple overlays enable the direct comparison of several data sets. DataWay can also be used offline to view saved data and to copy results to an Excel® file in just two clicks of the mouse.

■ Fluorimeter filters

Filter fluorimeters, such as the Jenway 62 series, use fixed filters to isolate both the excitation and emission wavelengths. The comparative ease of handling and the lower cost compared to scanning fluorimeters make it ideal for dedicated and routine measurements, particularly where sufficient chemistry has been carried out to eliminate interfering compounds. Optical filters are chosen to suit the requirements of each application. Often this choice will be a balance between the sensitivity and specificity requirements of the application.



Three types of optical filters are available from Jenway:

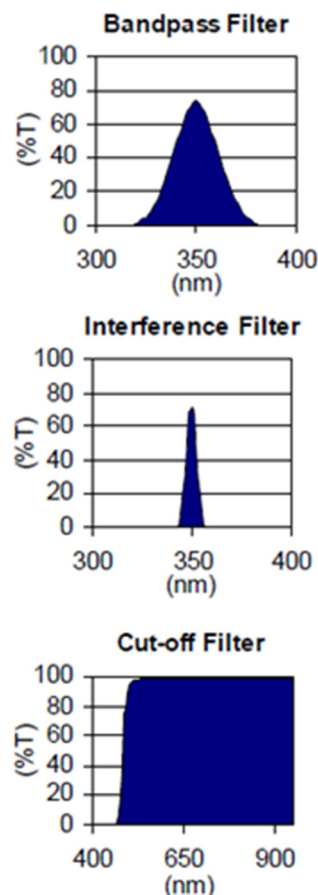
- **Bandpass** filters pass a broad band of light. For example, a 627 126 UG1 bandpass filter will transmit light from 320 - 380nm while blocking light with wavelengths shorter than 320nm and longer than 380nm.

- **Interference** filters pass a narrow band of light, typically 10nm. For example, a 627 140 - 350nm filter with a bandpass of 10nm will pass light from 345 - 355nm (5nm on either side of 350nm).
- **Cut-off** filters are used to block light that is shorter than a specified wavelength. A 475nm cut-off filter will allow transmission of light that is longer than 475nm, but it will block light that is shorter than 475nm.

An excitation filter should only allow light which excites the molecule of interest to reach the sample. The emission filter should allow the fluorescence from the sample to pass to the detector whilst blocking stray light from the light source or any interfering components in the sample.

Bandpass filters are often used for the excitation wavelengths in an application as they provide the required selectivity without compromising the sensitivity of the application. The emission filter is usually either an interference or cut-off type filter. These types of filter are able to isolate the fluorescence signal of interest from interferences that may be present in the sample. Using interference filters, with their narrow bandpass specification, may reduce the sensitivity of an application method; however the specificity is generally increased.

Bandpass and interference filters are made by coating optical glass with several thin layers of reflecting material. The number and arrangement of the layers determines the size of the bandpass region, the degree of transmission in that region and the degree of reflection outside that region. Bandpass and interference filters typically transmit between 10 and 70% of light and this is dependent on the wavelength and bandwidth.



Commonly used filter combinations

The following table lists various filter combinations and gives examples of which fluorophores can be used with them.

Excitation filter	Emission filter	Common fluorophores
UG1 320-380nm	460nm	Hoechst Dye, 4-methylumbelliferone (4-MU), 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP), quinine sulphate, NADH, histamine
480nm	520nm	Fluorescein, RiboGreen [®] reagent, PicoGreen [®] reagent, OliGreen [®] reagent, BODIPY FL.
470nm	590nm	NanoOrange [®] /Quant-iT [™] protein assay kit
550nm	580nm	Rhodamine WT, Phycoerythrin, Cy [®] 3
BG28 380-500nm	Kodak 29 610nm	Chlorophyll <i>a</i> (acidification method)
436nm	680nm	Chlorophyll <i>a</i> (non-acidification method)
620nm	650nm	Phycocyanin

Other applications

The table given below details some other applications which can be performed on filter based fluorimeters such as those of the Jenway 62 series. For each example, the given excitation and emission wavelengths are typical and for guidance only. Variations may occur due to the use of different methodologies and/or reagents and solvents. All experimental details should therefore be verified before analysis is undertaken. For this reason the recommended filter sets may not be optimal in every case.

Abbreviations: BP = band pass filter;
CO = cut-off filter;
IN = interference filter.

Application	Ex/Em (nm)	Excitation filter	Part code	Emission filter	Part code
Ethidium bromide	546/595	IN 546nm	627 176	IN 590nm	627 182
Rhodamine Green	502/527	BP BG28 380-	627 124	IN 530nm	627 173

		500nm or IN 500nm	627 170		
Rhodamine 6G	524/552	IN 520nm	627 172	IN 550nm	627 177
Rhodamine Red	560/580	IN 560nm	627 178	IN 580nm	627 181
Rhodamine	550/573	IN 550nm	627 177	IN 577nm	627 180
Coloured dissolved organic matter (DOM) (for high DOM concentrations use a low gain)	310-390/410-600	BP UG1 320-380nm	627 126	CO Kodak 2B 395nm	627 130
Ammonium assay	310-390/410-600	BP UG1 320-380nm	627 126	CO Kodak 2B 395nm	627 130
Aflatoxins					
B ₁ in chloroform	365/413	IN 365nm	627 138	IN 415nm	627 162
B ₁ in methanol	363/426	IN 365nm	627 138	IN 430nm	627 162
B ₁ in water, pH 2	370/435	IN 370nm	627 158	IN 436nm	627 165
B ₂ in chloroform	365/413	IN 365nm	627 138	IN 415nm	627 162
B ₂ in methanol	365/425	IN 365nm	627 138	IN 430nm	627 162
B ₃ in chloroform	365/432	IN 365nm	627 138	IN 430nm	627 162
G ₁ in chloroform	365/430	IN 365nm	627 138	IN 430nm	627 162
G ₁ in methanol	365/450	IN 365nm	627 138	IN 450nm	627 139
G ₂ in chloroform	365/430	IN 365nm	627 138	IN 430nm	627 162
G ₂ in methanol	365/450	IN 365nm	627 138	IN 450nm	627 139
Vitamins					
A in absolute ethanol	327/510	IN 326nm	627 153	IN 510nm	627 171
A in acetate	360/508	IN 360nm	627 157	IN 510nm	627 171
B ₁ Thiamine after oxidation to thiochrome	365/430-435	IN 365nm	627 138	IN 430nm	627 162
B ₂ Riboflavin	370/455-520	IN 370nm	627 158	CO Kodak 2B 395nm	627 130
B₆ compounds					
Pyridoxine	340/400	IN 340nm	627 133	IN 400nm	627 135
Pyridoxamine	335/400	IN 334nm	627 155	IN 400nm	627 135
Pyridoxal	330/385	IN 330nm	627 154	IN 390nm	627 159
B ₁₂	275/340	IN 280nm	627 145	IN 340nm	627 133
E α-tocopherol	295/340	IN 295nm	627 147	IN 340nm	627 133
p-Aminobenzoic acid	294/345	IN 295nm	627 147	IN 350nm	627 140
Folic acid	365/450	IN 365nm	627 138	IN 450nm	627 139

■ Other fluorophores

It is strongly urged that the data for a particular fluorophore be confirmed by consulting the dye manufacturer to secure full specification of curves for excitation and emission. Please be aware that shown maximum values of excitation and emission do not necessarily apply in every instance. If the operator uses another salt of the compound, or the pH value is different, excitation and emission values may vary greatly.

Fluorophore	Excitation (nm)	Emission (nm)	Comment
3-Hydroxypyrene 5,8,10-Tri Sulphonic acid	403	513	
5-Hydroxy Tryptamine (5-HT)	380-415	520-530	(Serotonin) Neurotransmitter
Acid Fuchsin	540	630	
Acridine Orange (bound to DNA)	502	526	Nucleic acid stain
Acridine Red	455-600	560-680	Nucleic acid stain
Acridine Yellow	470	550	pH indicator
Acriflavin	436	520	Antiseptic
AFA (Acriflavin Feulgen SITSA)	355-425	460	DNA stain
Alizarin Complexon	530-560	580	Binds fluoride
Alizarin Red	530-560	580	Calcium indicator
Allophycocyanin	650	661	Found in cyanobacteria
ACMA	430	474	pH indicator
Aminoactinomycin D	555	655	DNA stain
Aminocoumarin	350	445	Antibiotic
Anthroyl Stearate	361-381	446	Chloroplast membrane stain
Astrazon Brilliant Red 4G	500	585	

Astrazon Orange R	470	540	
Astrazon Red 6B	520	595	
Astrazon Yellow 7 GLL	450	480	
Atabrine	436	490	(Quinacrine) anti-malarial drug
Auramine	460	550	(Canary yellow) dye
Aurophosphine	450-490	515	
Aurophosphine G	450	580	
BAO 9-(Bisaminophenyloxadiazole)	365	395	
BCECF	505	530	pH indicator
Berberine Sulphate	430	550	Alkaloid from plant roots
Bisbenzamide	360	600-610	DNA stain
BOBO 1	462	481	Nucleic acid stain
Blancophor FFG Solution	390	470	Optical brightener
Blancophor SV	370	435	Optical brightener
Bodipy FI	503	512	Fluorescent tag
BOPRO 1	462	481	
Brilliant Sulphoflavin FF	430	520	
Calcein	494	517	Calcium indicator
Calcien Blue	370	435	Calcium indicator
Calcium Green	505	532	Calcium indicator
Calcium Orange	549	576	Calcium indicator
Calcofluor RW Solution	370	440	Optical brightener
Calcofluor White	440	500-520	Optical brightener
Calcophor White ABT Solution	380	475	Optical brightener
Calcophor White Standard Solution	365	435	Optical brightener
Cascade Blue	400	425	Fluorescent tag
Catecholamine	410	470	Hormone
Chinacrine	450-490	515	Chromatin stain
Coriphosphine O	460	575	RNA stain
Coumarin-Phalloidin	387	470	Actin stain
Cy3.18	554	568	Fluorescent tag
Cy5.18	649	666	Fluorescent tag
Cy7	710	805	Fluorescent tag
DANS (1-Dimethyl Amino Naphaline-5-Sulphonic Acid)	340	525	
DANSA (Diamino Naphthyl Sulphonic Acid)	340-380	430	
Dansyl NH-CH ₃ in water	340	578	
DAPI	350	470	Nucleic acid stain
Diamino Phenyl Oxydiazole (DAO)	280	460	
Dimethylamino-5-Sulphonic acid	310-370	520	
Diphenyl Brilliant Flavine 7GFF	430	520	Cellulose stain
Dopamine	340	490-520	Neurotransmitter
Eosin	525	545	Cytoplasm, collagen and muscle fibre stain
Erythrosin ITC	530	558	
Ethidium Bromide	510	595	Nucleic acid stain
Euchrysin	430	540	
FIF (Formaldehyde Induced Fluorescence)	405	435	Biogenic amine analysis
Flazo Orange	375-530	612	
Fluorescein	494	518	(Uranine) dye
Fluorescein Isothiocyanate (FITC)	490	525	Protein labelling
Fluo-3	485	503	Calcium indicator
Fura-2	340-380	505-512	Calcium indicator
Genacryl Brilliant Red B	520	590	Polyester dye
Genacryl Brilliant Yellow 10GF	430	485	
Genacryl Pink 3G	470	583	Polyester dye
Genacryl Yellow 5GF	430	475	
Gloxalic Acid	405	460	
Granular Blue	355	425	Cellular tracer dye
Haematoporphyrin	530-560	580	Artificial Porphyrin
Hoechst 33258, 33342 (bound to DNA)	352	461	DNA stain
Indo-1 350	350	405-482	Calcium indicator

Intrawhite Cf Liquid	360	430	Optical brightener
Leucophor PAF	370	430	Optical brightener
Leucophor SF	380	465	
Leucophor WS	395	465	Optical brightener
Lissamine Rhodamine B200 (RD200)	575	595	Protein stain
Lucifer Yellow CH	425	528	Nerve cell stain
Lucifer Yellow VS	430	535	Protein stain
Magdala Red	524	600	Detection of yeast mutants
Maxilon Brilliant Flavin 10 GFF	450	495	
Maxilon Brilliant Flavin 8 GFF	460	495	
MPS (Methyl Green Pyronine Stilbene)	364	395	
Mithramycin	450	570	Antibiotic used to reduce calcium levels
NBD Amine	450	530	Fluorescent tag
Nile Red	515-530	525-605	Lipid and hydrophobic probe
Nitrobenzoxadidole	460-470	510-650	
Noradrenaline	340	490-520	Hormone
Nuclear Fast Red	289-530	580	Calcium indicator
Nuclear Yellow	365	495	
Nylosan Brilliant Flavin E8G	460	510	
Oregon Green 488	496	524	Fluorescent tag
Oregon Green 500	503	522	Fluorescent tag
Oregon Green 514	511	530	Fluorescent tag
Pararosaniline (Feulgen)	570	625	Used in Schiff's reagent
Phorwite AR Solution	360	430	Optical brightener
Phorwite BKL	370	430	Optical brightener
Phorwite Rev	380	430	Optical brightener
Phorwite RPA	375	430	Optical brightener
Phosphine 3R	465	565	Determination of fat in emulsions
Phycoerythrin R	480-565	578	Protein used for cross-linking
Pontochrome Blue Black	535-553	605	
Primuline	410	550	Dye for cotton
Procion Yellow	470	600	Dye for cotton
Propidium Iodide	536	617	Nucleic acid stain
Pyronine	410	540	Nucleic acid stain
Pyronine B	540-590	560-650	Biological stain
Pyrozal Brilliant Flavin 7GF	365	495	
Quinacrine Mustard	423	503	Chromosome stain
Rhodamine 110	496	520	Fluorescent tag
Rhodamine 123	511	534	Inhibits mitochondrion function
Rhodamine 5 GLD	470	565	
Rhodamine 6G	526	555	Tracer dye
Rhodamine B	540	625	Tracer dye
Rhodamine B 200	523-557	595	
Rhodamine B Extra	550	605	
Rhodamine BB	540	580	
Rhodamine BG	540	572	
Rhodamine Green	502	527	Fluorescent tag
Rhodamine Red	570	590	Fluorescent tag
Rhodamine WT	530	555	Tracer dye
Rose Bengal	540	550-560	Stain used in eye drops to detect damage
Serotonin	365	520-540	Neurotransmitter
Sevron Brilliant Red 2B	520	595	
Sevron Brilliant Red 4G	500	583	
Sevron Brilliant Red B	530	590	
Sevron Orange	440	530	
Sevron Yellow L	430	490	
SITS (Primuline)	395-425	450	
SITS (Stilbene Isothiosulphonic acid)	365	460	
Sodium Green	507	535	Sodium indicator
Stilbene	335	440	Optical brightener

Snarf 1	563	639	pH indicator
Sulphorhodamine B Can C	520	595	Tracer dye
Sulphorhodamine G Extra	470	570	Tracer dye
SYBR Green I	498	522	DNA stain
SYTO Green fluorescent nucleic acid stains	494±6, 515±7	515±7, 543±13	Nucleic acid stain
SYTOX Green nucleic acid stain	504	523	Nucleic acid stain
Tetracycline	390	560	Antibiotic
TRITC (Tetramethyl Rhodamine Isothiocyanate)	557	576	
Texas Red	596	615	Fluorescent tag
Thiazine Red R	510	580	
Thioflavin S	430	550	Derivative of Primuline
Thioflavin TCN	350	460	
Thioflavin 5	430	550	
Thiolite	370-385	477-484	Fluorescent tag
Thiozol Orange	453	480	
Tinopol CBS	390	430	Optical brightener
TOTO 1, TO-PRO-1	514	533	DNA stain
TOTO 3, TO-PRO-3	642	661	DNA stain
True Blue 365	365	420-430	
Ultralite	656	678	
Uranine B	420	520	(Fluorescein) Tracer dye
Uvitex SFC	365	435	Optical brightener
Xylene Orange	546	580	
XRITC	582	601	
YOYO-1, YO PRO 1	491	509	DNA stain

■ Application notes and protocols

Below is a brief summary of the application notes currently available for the Jenway 62 series fluorimeters. The notes can be downloaded in full from http://www.jenway.com/notes_Fluorimeters.asp or obtained by contacting jenwayhelp@bibby-scientific.com.

• A10-001A: Quant-iT™ OliGreen® ssDNA assay

The Quant-iT™ OliGreen® oligonucleotide reagent is a sensitive fluorescent nucleic acid stain for quantitating oligonucleotides and single stranded DNA (ssDNA) in solution. Alternative methods for quantitating oligonucleotides, such as spectrophotometry, often require large sample volumes or a highly concentrated sample and are only sensitive to around 1µg/ml. In addition, free nucleotides can contribute significantly to the signal as can contaminants often found in nucleic acid preparations. Fluorimetric assays such as the Quant-iT™ OliGreen® assay can overcome these issues and improve sensitivity by several orders of magnitude. We describe the use of the Jenway model 6285 fluorimeter for performing the Quant-iT™ OliGreen® assay and quantitating oligonucleotide primers for DNA amplification.

• A10-002A: Quantitation of dsDNA using Hoechst 33258

Hoechst 33258 is a bisbenzimidazole dye which binds specifically to AT-rich regions of double-stranded DNA. On binding, the dye exhibits a large increase in fluorescence coupled with a shift in the emission maximum wavelength from 492nm to 458nm. The excitation wavelength remains the same at 356nm. Since it is an intercalating dye, the assay is not normally affected by short lengths of single-stranded DNA or RNA. In addition, proteins, high salt concentrations or low levels of detergent have little effect. We show that using the Jenway model 6285 fluorimeter, the sensitivity of the assay is approximately 5ng/ml and that at up to 20µg/ml can be detected, a dynamic range of more than 4 orders of magnitude.

• A10-003A: Fluorimetric Determination of Acetylsalicylic Acid in Aspirin

Acetylsalicylic acid (ASA) is the analgesic compound found in aspirin tablets. In addition to its pain relieving properties, it is also used as an antipyretic and anti-inflammatory. It also has an anticoagulant effect and is used long term in low doses to prevent heart attacks. In the presence of sodium hydroxide, ASA is converted to salicylate ions which fluoresce strongly at around 400nm when excited at about 310nm. We describe the extraction of ASA from commercially available aspirin tablets and measurement of salicylate ion fluorescence. The concentration of ASA extracted from each tablet was calculated by comparison with

standards prepared from salicylic acid (SA) and the results assessed against the requirements of the British Pharmacopoeia.

- **A10-004A: NanoOrange® protein quantitation assay**

The NanoOrange® Protein Quantitation Kit is a sensitive fluorescent assay for detecting proteins in solution. The NanoOrange® reagent is virtually non-fluorescent in aqueous solution but on interaction with proteins, exhibits a strong fluorescence at about 570nm when excited at around 470nm. The assay is performed simply by adding the protein sample to the diluted reagent; heating at 95°C for ten minutes and reading the fluorescence once the sample has cooled to room temperature. Using the model 6285 fluorimeter, protein concentrations as low as 100ng/ml can be detected, which is a considerable increase in sensitivity compared to common spectroscopic protein determination methods.

- **A10-005A: Test of fluorescent dye dilutions and limit of detection**

To test the sensitivity of the 6285 fluorimeter, four oligonucleotides labelled with different fluorescent dyes were diluted and the fluorescence measured.

- **A10-006A: Determination of chlorophyll a using fluorimetry**

The photosynthetic pigment chlorophyll is present in most plants, algae and cyanobacteria. It can be measured both by spectrophotometry and fluorimetry as an indicator of the abundance of photosynthetic organisms in fresh and salt water. In fresh water, levels of chlorophyll are also an important factor in determining water quality. Chlorophyll pigments may be present in several forms in varying ratios, the most common being chlorophyll *a*. In this application note we demonstrate two methods of determining chlorophyll *a* using the Jenway 6285 fluorimeter based on EPO method 445.0.

- **A10-007A: Phosphatase assays using DiFMUP as substrate**

Phosphatases are enzymes that remove phosphate groups from a substrate. De-phosphorylation may either activate or deactivate an enzyme, resulting in an increase or decrease in activity of a particular biochemical pathway. In this application note we demonstrate use of the EnzChek® Phosphatase Assay Kit which contains the artificial substrate, 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP). DiFMUP can be used at neutral, acidic and alkaline pH. Using this kit with the Jenway Model 6285 fluorimeter we demonstrate activity of both acid phosphatases (potato and prostatic) and calf intestinal alkaline phosphatase simply by changing the reaction buffer and pH conditions.

- **A10-008A: Limit of detection determination using quinine sulphate dihydrate**

Quinine is a very strongly fluorescing compound, especially in dilute acid solution and therefore very low concentrations can be detected. For this reason it is often used to determine the sensitivity of a fluorimeter by measuring the limit of detection (LOD). The basis for quantitation is that the intensity of fluorescence emission in very dilute solutions is directly proportional to the concentration of quinine provided the intensity of the excitation source and other experimental factors are kept constant. In this application note the LOD of quinine is determined and the sensitivities of the models 6270 and 6285 are compared.

- **A10-009A: The effect of emission filter selection on LOD determination using quinine sulphate dihydrate**

Using a similar method to A10-008A, in this application note the LOD of quinine is determined using three different types of emission filter to show the effect of emission filter selection on the LOD. The sensitivity of the model 6270 fluorimeter with a silicon diode detector and the model 6285 with a PMT is also compared.

- **A10-010A: Kinetics of hexokinase measured using the fluorescence of NADH**

Measurement of enzyme activity requires the determination of the amount of product produced or the disappearance of substrate consumed. With many assays this cannot always be done directly, therefore the reaction may be coupled to a second enzyme that can convert one of the products into a measurable substance. One such example is the coupling of reactions to dehydrogenases that use nicotinamide adenine dinucleotide (NAD⁺) or the reduced form, NADH, as coenzymes. NADH can be readily measured in a spectrophotometer at 340nm or by fluorimetry with excitation at 340nm and emission at 460nm.

- **A10-011A: The Effect of Filter Selection on the LOD in the Fluorometric Determination of Histamine in Seafood**

Histamine is a biogenic amine that can be formed in food when histidine is converted to histamine by the metabolic processes of microorganisms. Consumption of foods that contain high levels of biogenic amines can result in an illness called histamine poisoning. This application note shows the use of different combinations of narrow and wide bandpass excitation and emission filters to determine the concentration of histamine corresponding to the LOD. The application note also considers the relative sensitivity and suitability of the models 6270 and 6285 when performing the AOAC Histamine in Seafood methodology.

- **P10-001A: Quant-iT™ OliGreen® ssDNA assay**

A method for quantitating single stranded DNA, including oligonucleotide primers for DNA amplification, using the Quant-iT™ OliGreen® oligonucleotide reagent from Invitrogen Ltd (Product O7582).

- **P10-002A: Quantitation of dsDNA using Hoechst 33258**

A method for quantitating double stranded DNA using the intercalating fluorescent dye Hoechst 33258.

- **P10-003A: Fluorimetric determination of acetylsalicylic acid in aspirin**

A method for extraction of acetylsalicylic acid from commercially available aspirin tablets followed by its conversion to fluorescent salicylate ions.

- **P10-004A: NanoOrange® protein quantitation assay**

A fluorimetric method for quantitating protein using the NanoOrange® Protein Quantitation Kit from Invitrogen Ltd (Product N6666).

- **P10-005A: Determination of chlorophyll *a* using fluorimetry**

A method for determination of chlorophyll *a* as described in EPO method 445.0.

- **P10-006A: EnzChek® Phosphatase assay**

A method for measuring activity of neutral, acid and alkaline phosphatases using the EnzChek® Phosphatase Assay Kit (E12020) from Invitrogen which contains the fluorescent artificial substrate, 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP).

- **T10-001: An Overview of the 62 Series Fluorimeters Optical Filters**