

Tech. Note #19

FLUORESCENT MICROSPHERES

Bangs Laboratories presents fluorescent microspheres with rich colors spanning the spectrum from brilliant blues to vibrant reds. With our understanding of the special requirements for various applications, we can create and match colors and dye intensities to the satisfaction of many successful customers – large and small.

Our fluorescent microspheres excite and fluoresce at wavelengths corresponding to standard filter sets: coumarin (blue), fluorescein (yellow-green), rhodamine (orange), and Texas Red® (red). Familiar dyes, such as fluorescein, acridine orange, DCM, rhodamine and propidium iodide, are closely approximated by our dyed microspheres, to give you a full range of choices.

If your application requires fluorescent microspheres with spectral properties not covered by our standard products, please contact us. We will custom dye the microspheres of your choice to meet your specifications.

Colors of the Visible Spectrum

<u>Wavelength (nm)</u>	<u>Color Absorbed</u>
< 400	Ultra-Violet
400-424	Violet (410 nm)
424-491	Blue (470 nm)
491-575	Green (520 nm)
575-585	Yellow (580 nm)
585-647	Orange (600 nm)
647-700	Red (650 nm)
> 700	Infrared

Data from the CRC Handbook of Chemistry and Physics, 54th Edition, 1973 - 1974.

Some Common Dyes

<u>Name</u>	<u>Excitation Max.</u>	<u>Emission Max.</u>
DAPI	344	450
Fluorescein	492nm	519nm
Rhodamine	499	521
Acridine Orange	500	526
R-Phycoerythrin	500,545, <u>565**</u>	578
Propidium Iodide	535	617
Texas Red*	587	602

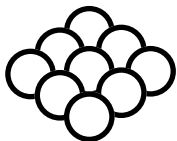
*Texas Red is a trademarked product of Molecular Probes Inc.

**Underlined value indicates maximum excitation wavelength.

Conventional Fl. Excitation Sources

<u>Sources</u>	<u>Useful Wavelengths (nm)</u>	<u>Principal Lines (nm)</u>
Argon	350, 360, 458, 476, 488, 514	488, 496, 514
He-Cd	325, 442	442
He-Ne	543, 594, 633	633
Kr	530, 568, 647, 676	647
Tungsten Filamt.	350–1000	—
Mercury Arc	250–600	254, 366, 436, 546
Xenon Arc	250–1000	467

Only primary excitation sources capable of continuous operation have been considered. Several other laser sources are available that either provide pulsed output (e.g. N₂ laser) or require pumping (e.g. dye lasers, Ti: Sapphire lasers).¹



Applications

Medical Diagnostics

Immunoassays^{2,3,4}
DNA Hybridization Probes⁵
Flow Cytometry^{6,7,8}

Tracers to study:

Phagocytic Processes⁹
Environmental Pollution

Standards for:

Flow Cytometers
Microscopes (Including Confocal)
Centrifuges
Light Scattering Instruments
Filtration Equipment

Others

Antibody Labeling (dyed 20 nm)^{10,11,12}
Biosensors¹³
Components of Model Systems¹⁴

Confocal Standards (63 nm diameter)

Name	Excitation	Emission
PS• (360,420)	360, 380nm	420 nm
PS• (555,570)	530, 555	570
PS• (660,690)	660	690

These materials were designed to be used as point spread function calibrators for confocal microscopes.

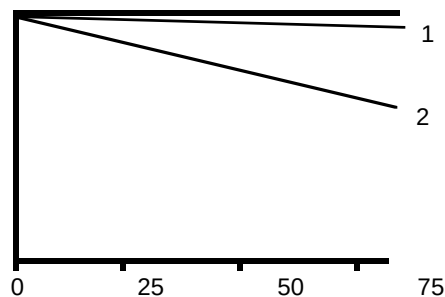
Fluorescent Microsphere Characteristics

Fluorescence is a molecular phenomenon in which the absorption of photons by a fluorescent material energizes electrons from a ground state to a higher energy (excited) state, and almost immediately, emits a photon of light by the return of those electrons to a lower energy state. Organic dyes which exhibit this property are called **fluorochromes**.

A particular dye's **Stokes shift** is the difference between its excitation maximum and its emission maximum.

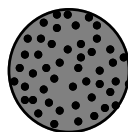
To create fluorescent microspheres, chemists either covalently attach specific dyes to functional groups on the microsphere surface, or absorb dyes with special solubility properties into the microsphere interior. Our techniques for absorbing fluorochromes into the interior allow us to create brighter beads which are

better protected from photolytic degradation and demonstrate longer shelf lives.¹⁵

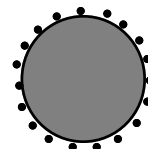


Elapsed time (minutes)
Relative photobleaching rates of latex-bound dye (1) and free dye (2).⁹

The figures below illustrate that a great many more dye molecules can be internalized within a microsphere, than can be covalently bound to the surface. In addition to greater dye loading (more color) with internalized fluorochromes, the chemical functional groups remain intact and are available to bind ligands.



Microsphere
with Dye
Throughout



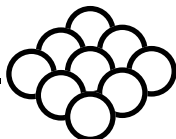
Microsphere
with Dye Bound
to Surface

Notes on Our Nomenclature

In the attached listing of our products, the "Polymer Description" section describes the polymeric make-up of the microspheres, as well as the maximum excitation and emission wavelengths. [PS•(555,570) excites at 555nm and emits at 570nm]

Many of these microspheres have secondary excitation and emission peaks, so you are encouraged to refer to the enclosed spectra when determining which beads are appropriate for use with your laser.

The "Color" section describes the visual color of the dyed microsphere suspension. Please note that visual color and fluorescent color are not always the same. For example, PS•(660,690) fluoresces in the red, but the microsphere suspension appears blue.



estapor® Microspheres Detection Level Limit

The detection level limit is the minimum concentration of fluorescent particles (# of microspheres per liter suspension) where the emission signal is 10 times the background signal. This is measured under standard conditions: Xenon X BO 150 W lamp, 2 x 1/4 m monochromater; Hammamatsu R928 PMT; Keithley 6/0 C electrometer.

Dye Name	*Excitation Wavelength(s)	*Emission Wavelength(s)	Size Range (microns)	Surface Function (µeq COOH/ g)	Detection Level Limit (# of microspheres / L)
(397,409) U estapor®	358,377, <u>397</u> nm	<u>409</u> ,430nm	0.050-0.195 0.275-0.325 0.275-0.325 0.750-0.850 3.500-4.500	400-520 ---- 220-450 3-10 OH	Scintillators
(460,480) X estapor®	445, <u>460</u>	<u>480</u> ,510	0.050-0.195 0.050-0.195 0.275-0.325 0.275-0.325 0.750-0.850	---- 400-520 ---- 220-450 3-10	9.03 x 10 ¹⁰ 3.61 x 10 ⁹ 2.41 x 10 ⁹ 4.21 x 10 ⁸ 4.21 x 10 ⁷
(480,520) XC estapor®	<u>480</u> ,500	520	0.050-0.195 0.275-0.325 0.275-0.325 0.750-0.850	400-520 ---- 220-450 3-10	9.63 x 10 ⁹ 1.81 x 10 ⁹ 9.03 x 10 ⁸ 9.03 x 10 ⁷
(555,570) Y estapor®	480,515, <u>555</u>	<u>565</u> ,610	0.050-0.195 0.275-0.325 0.275-0.325 0.750-0.850	400-520 ---- 220-450 3-10	1.20 x 10 ¹⁰ 9.03 x 10 ⁹ 4.21 x 10 ⁹ 3.01 x 10 ⁸
(broad,656) Z estapor®	Broad	<u>600</u> ,650	0.050-0.195 0.275-0.325	400-520 220-450	2.05 x 10 ¹² 1.20 x 10 ¹¹

*Please note that underlined values indicate excitation and emission maxima.

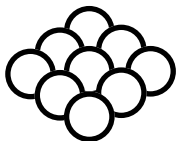
References

- ¹Brinkley M. , et al, (1994) U.S. Patent 5,326,692, "Fluorescent Microparticles with Controllable Enhanced Stokes Shift."
- ²McHugh T.M., Wang Y.J., Chong H.O., Blackwood L.C. and Stites D.P. (1989) Development of a microsphere-based fluorescent immunoassay and its comparison to an enzyme immunoassay for the detection of antibodies to three antigen preparations from *Candida albicans*. *J. Immunol. Methods* 116, 213.
- ³Lisi P.J., Huang C.W., Hoffman R.A. and Teipel J.W. (1982) A fluorescent immunoassay for soluble antigens employing flow cytometric detection. *Clin. Chim. Acta* 120, 171.
- ⁴Cook P., Linda and D. Irvng, (1989) Microsphere-based Flow Cytometric Assays, *Journal of Clinical Immunoassay*, 12 ,36.
- ⁵Cardullo R.A., Sughin A., Flores L., Zamecnik P.C. and Wolf D.E. (1988) Detection of nucleic acid hybridization by nonradiative fluorescence energy transfer. *Proc. Natl. Acad. Sci. U.S.A.* 85, 8790.
- ⁶Reinhardt, J., Balcerzak S. and Lobuglio A. (1967) Study of the malaria-red cell relationship with the use of a new immunologic marker. *J. Lab. Clin. Med.* 78, 167.
- ⁷Rembaum A. (1979) Microspheres as immunoreagents for cell identification. *Flow Cytometry and Sorting*, pp 335-347, Melmud M.R., Mullaney P.F. and Mendelsohn M.L. (eds), Wiley-Liss, Inc., New York.
- ⁸Fatigati V. and Peach M. (1988) Development of a new fluorescent angiotensin II probe. *Am. J. Physiol.* 255, C452.

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⁹Falk L.A. and Vogel S.N. (1988) Comparison of bone marrow progenitors responsive to granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor-I. *J. Leukocyte Biol.* 43, 148.

¹⁰Brinkley M. (1996) Diagnostic applications of fluorescent latex microspheres. *The Latex Course*, April, 1996, Princeton, New Jersey.

¹¹Jensen B.D., (1994) Immunoassay for Determination of Cells, in Zynaxis, Inc., US Patent 5,374,531.

¹²Molday R.S., *et al.*, (1975) New Immunolatex Spheres: Visual Markers of Antigens On Lymphocytes for Scanning Electron Microscopy. *The Journal of Cell Biology*, 64, 75.

¹³Alvager T., *et al.*, (1991) Applications of Fluorescent Biosensor to Cell Culture Technology, *American Laboratory*, November, 21.

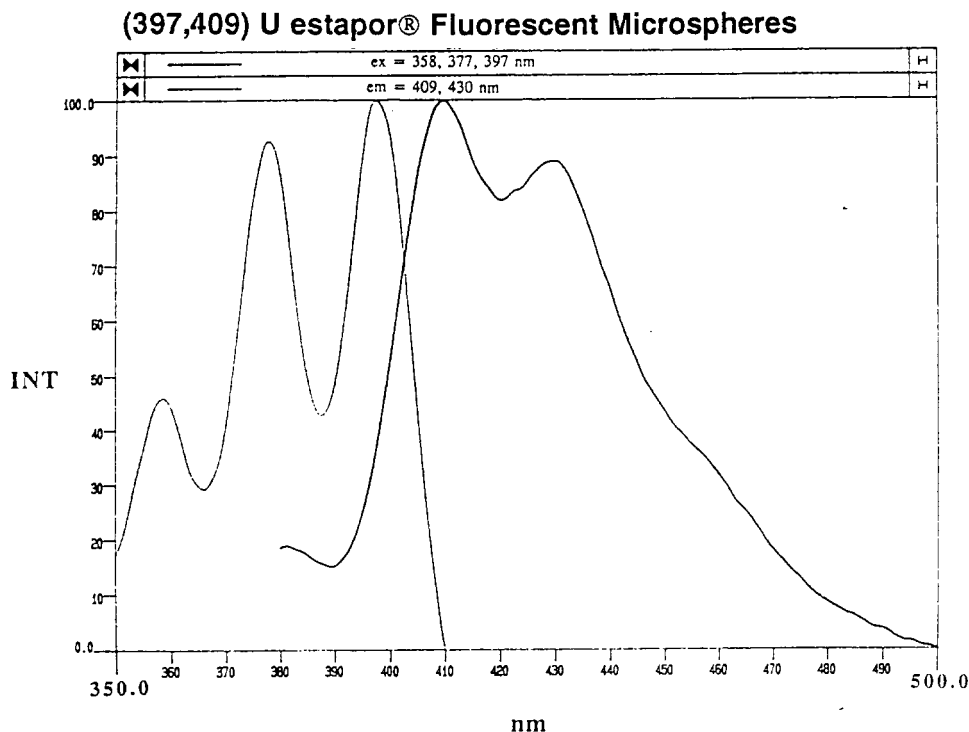
¹⁴Vrolijk J., *et al.*, (1994) Applicability of a Noncooled Video-Rated CCD Camera for Detection of Fluorescence In Situ Hybridization Signals, *Cytometry*, 15, 2.

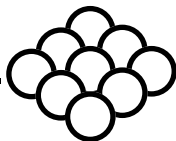
¹⁵Hook G. and Odeyale C.O. (1989) Confocal scanning fluorescence microscopy: a new method for phagocytosis research. *J. Leukocyte Biol.* 45, 277.

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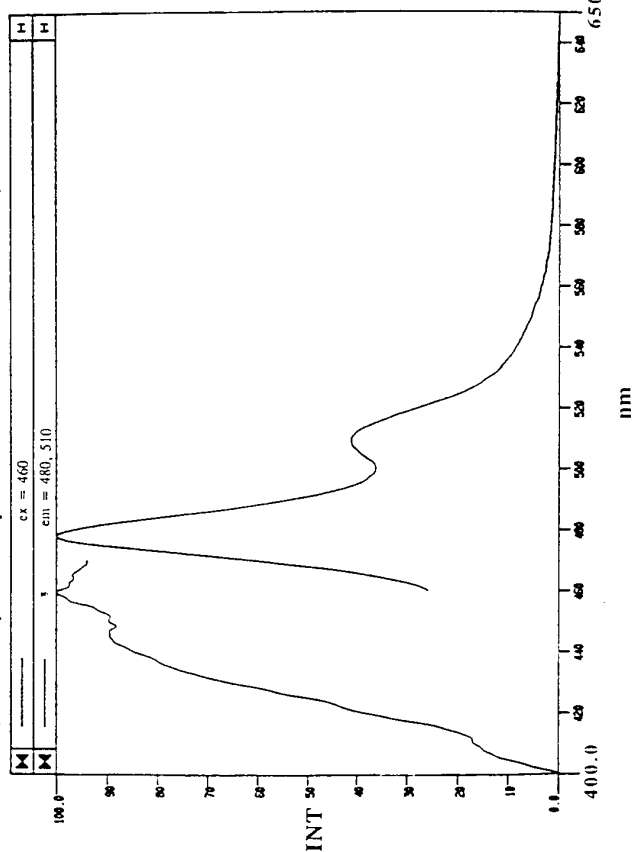
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estapor® Fluorescent Microspheres

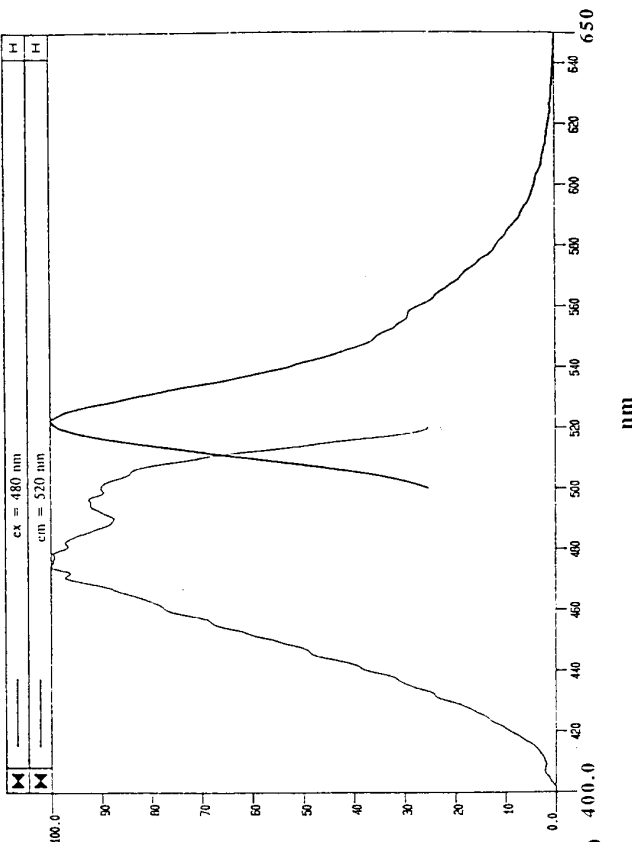




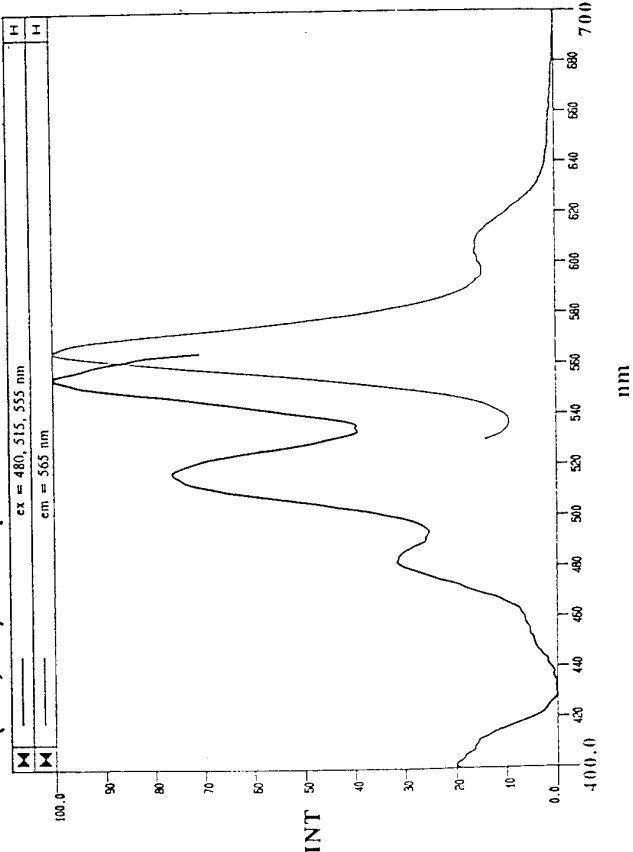
(460,480) X estapor® Fluorescent Microspheres



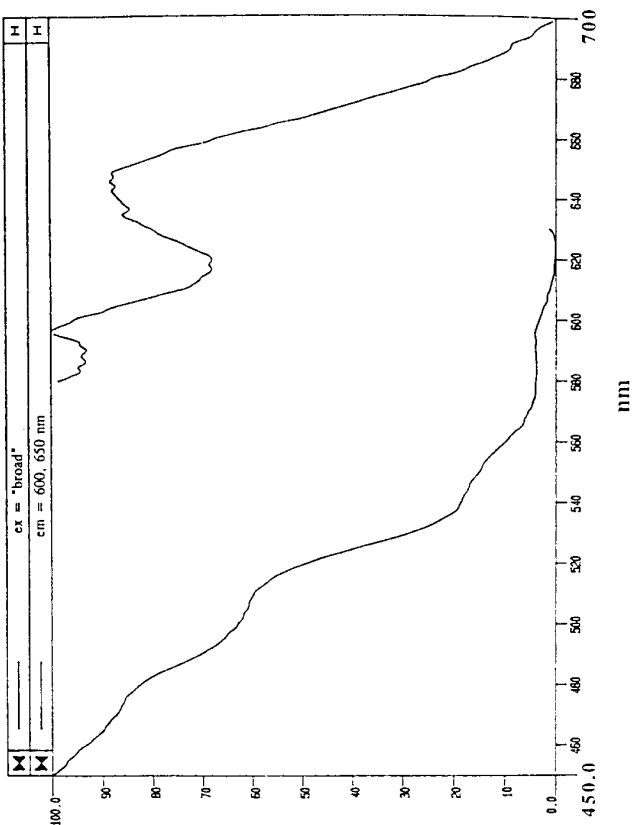
(480,520) XC estapor® Fluorescent Microspheres

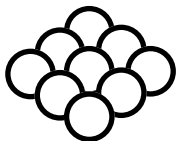


(555,570) Y estapor® Fluorescent Microspheres

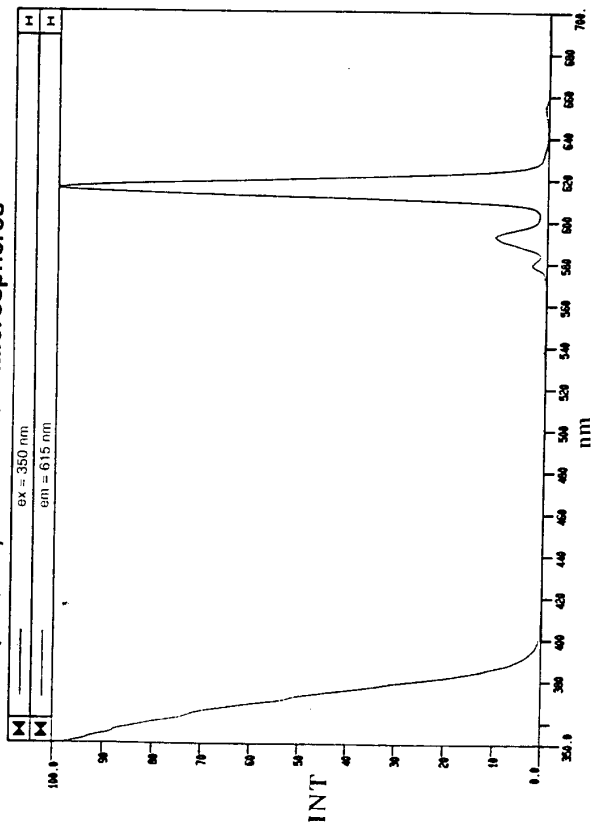


(broad, 656) Z estapor® Fluorescent Microspheres

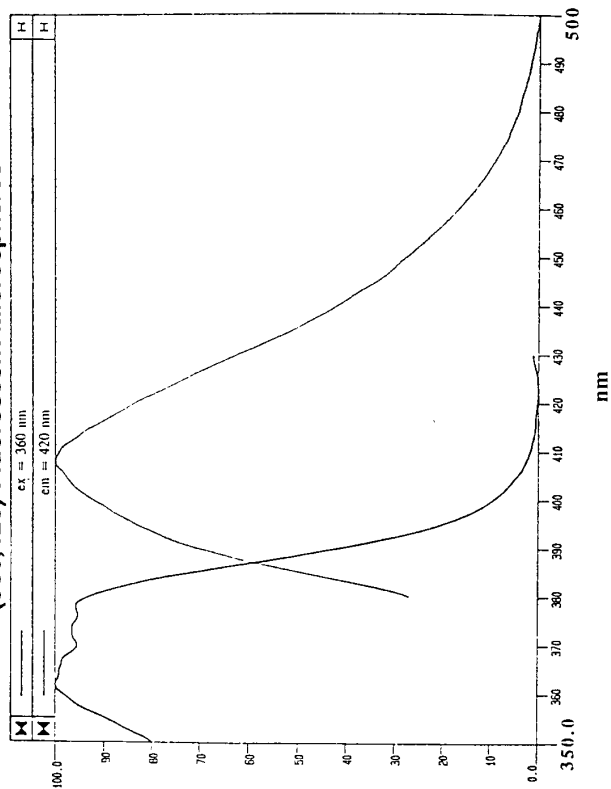




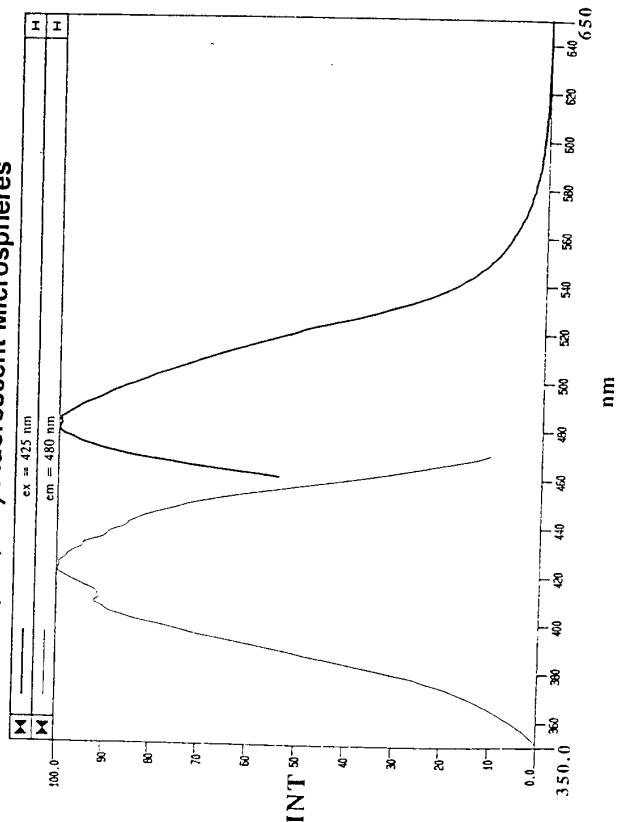
(350,615) Fluorescent Microspheres



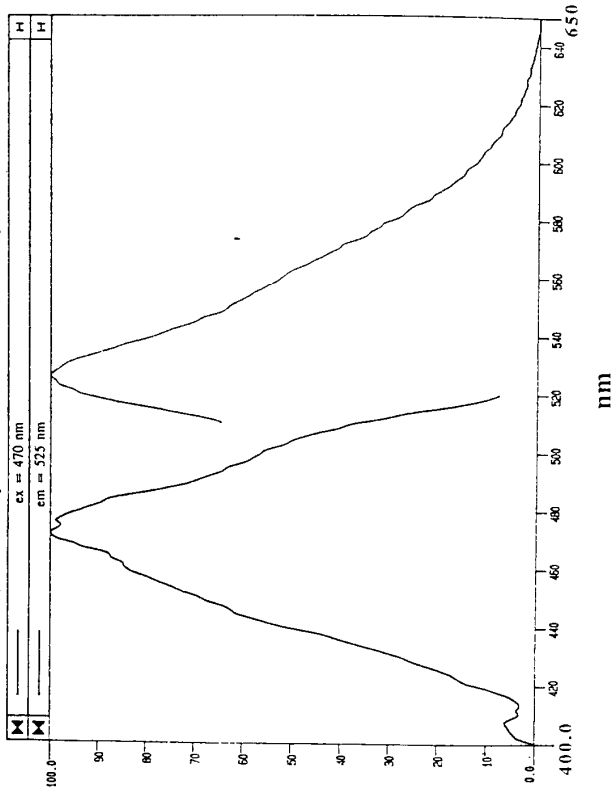
(360,420) Fluorescent Microspheres

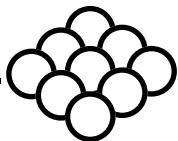


(425,480) Fluorescent Microspheres

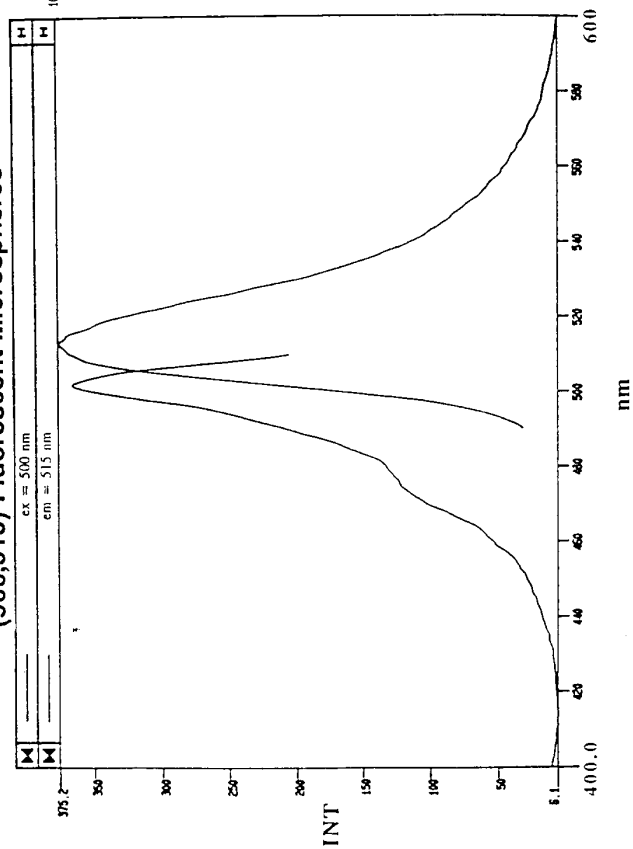


(470,525) Fluorescent Microspheres

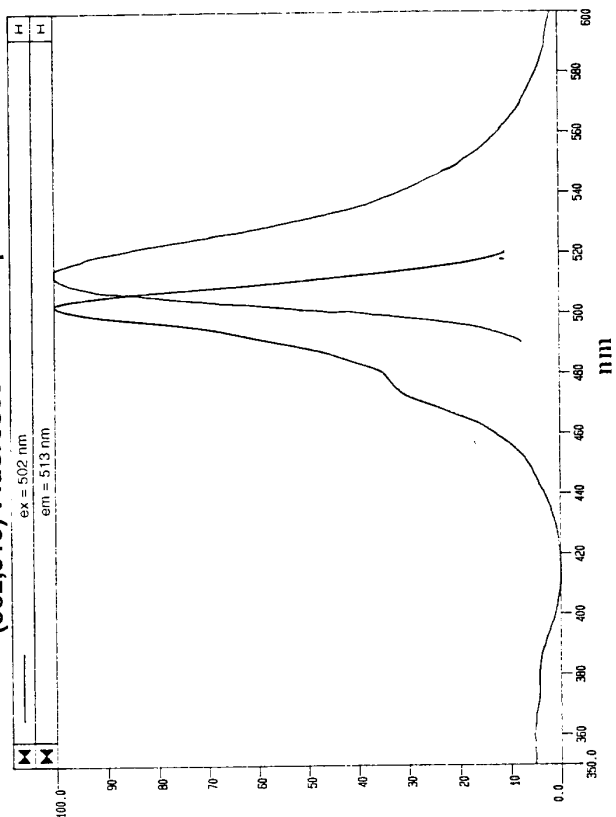




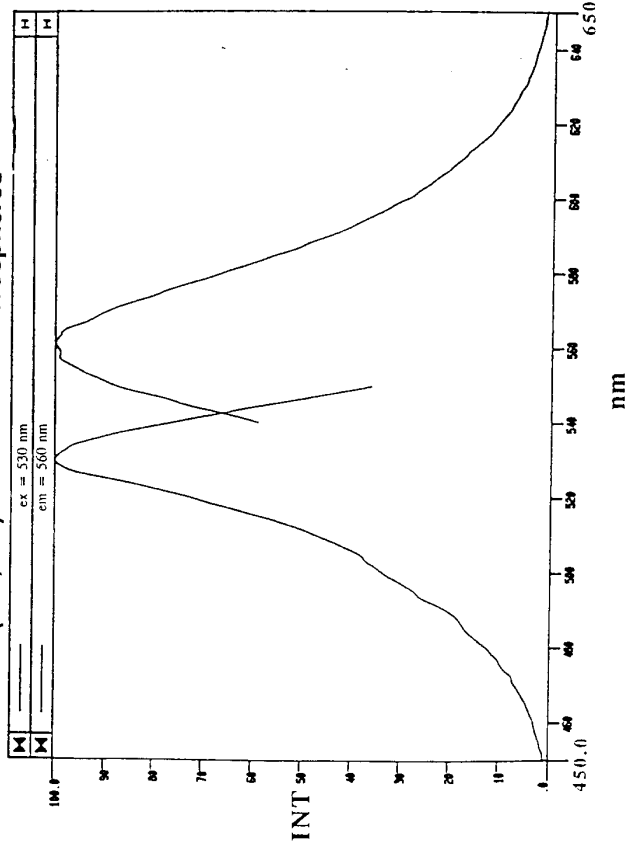
(500,515) Fluorescent Microspheres



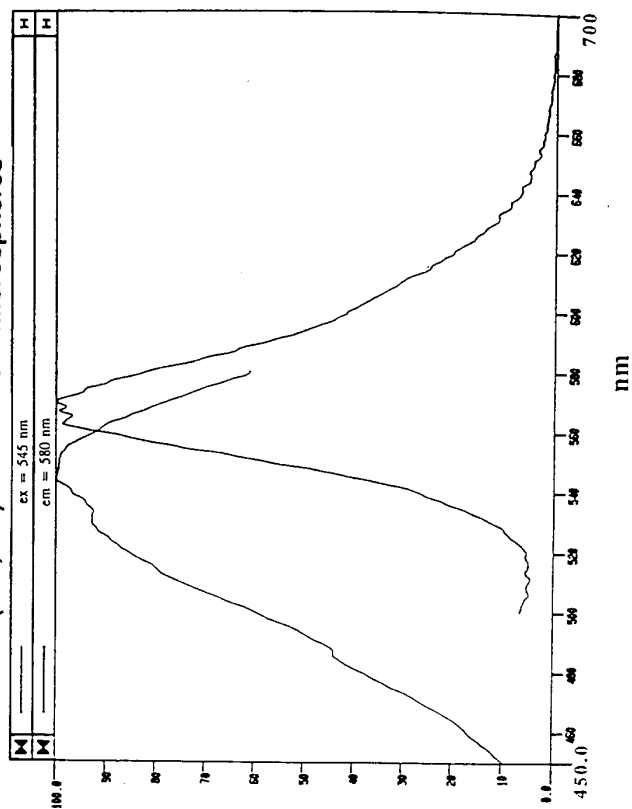
(502,513) Fluorescent Microspheres

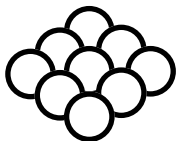


(530,560) Fluorescent Microspheres

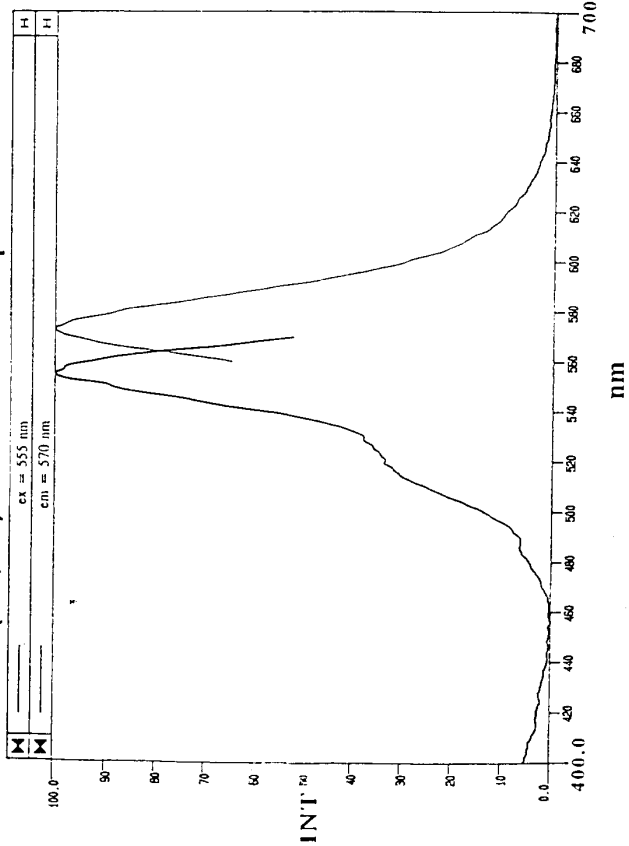


(545,580) Fluorescent Microspheres

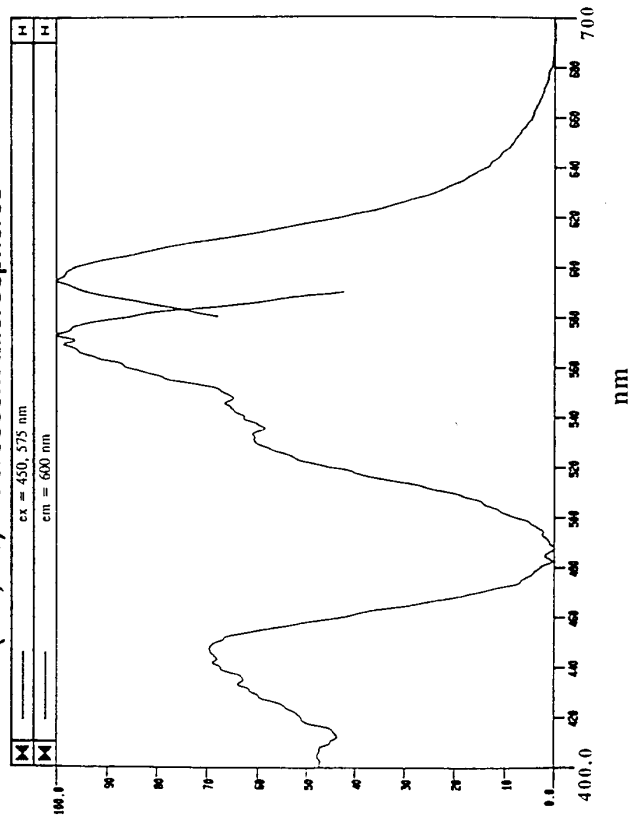




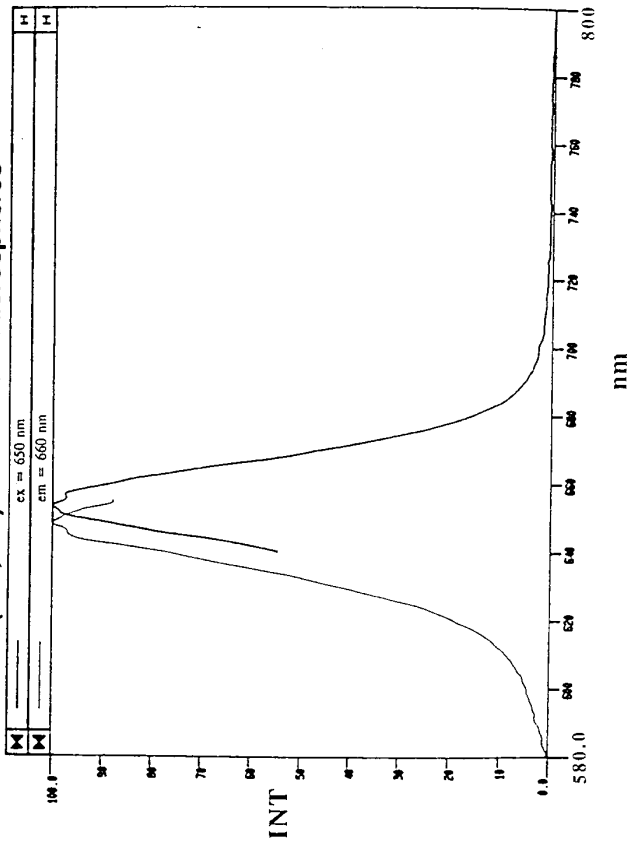
(555,570) Fluorescent Microspheres



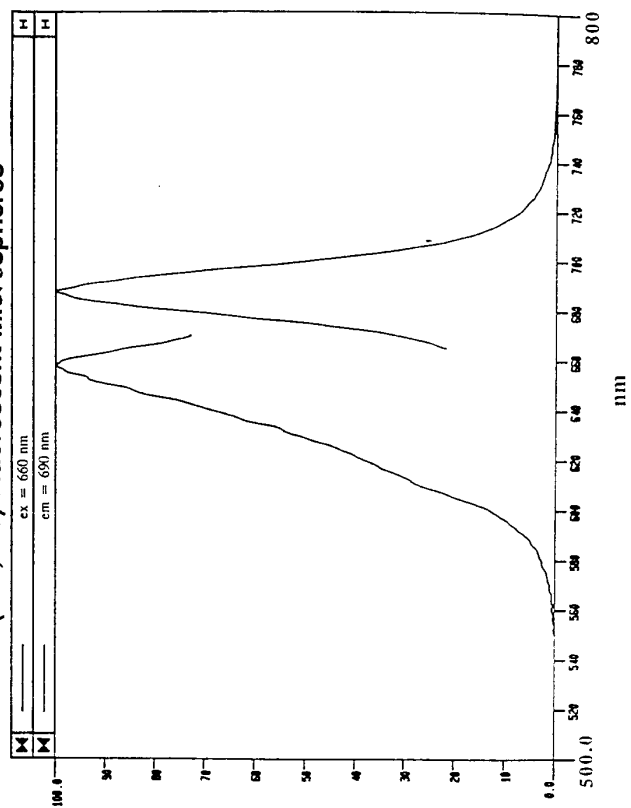
(575,600) Fluorescent Microspheres



(650,660) Fluorescent Microspheres



(660,690) Fluorescent Microspheres



Bangs Laboratories, Inc. Product List

1/15/98

FLUORESCENT MICROSPHERES

Stock Number	Nominal Mean Diameter (μm)	Std Dev (μm)	Polymer Description†	Color	Surface Groups	Surf. Charge (μeq/g)	Parking Area (sq.Å /grp)	% Solids	Price Sch.
Non-Functionalized									
• D0000630PF	0.06		PS•(360,420) {confocal}	Fl. White				1.0	Z
• D0000631PF	0.06		PS•(555,570) {confocal}	Fl. Pink				1.0	Z
• D0000632PF	0.06		PS•(660,690) {confocal}	Fl. Blue				1.0	Z
• D0000633PF	0.06		PS•(650,660)	§ Fl. Blue				5.0	E
D0000980PF	0.10	0.01	PS•(460,480)X estapor®	Fl. Yellow				1.0	T
• D0001000PF	0.10	0.01	PS•(500,515)	§ Fl. Yel-Orn				10.0	D
D0002800PF	0.28	0.01	PS•(397,409)U estapor®	Fl. White				1.0	T
• D0002950PF	0.30	0.01	PS•(460,480)X estapor®	Fl. Yellow				1.0	T
• D0002951PF	0.30	0.01	PS•(555,570)Y estapor®	Fl. Pink				1.0	T
D0003142PF	0.31		PS•(480,520)XC estapor®	Fl. Yel-Grn				1.0	T
• D0020001PF	2.00		PS•(575,600)	Fl. Pink				2.5	T
D0020300PF	2.03		PS•(480,520)XC estapor®	Fl. Yel-Grn				1.0	T
D0020301PF	2.03		PS•(555,570)Y estapor®	Fl. Pink				1.0	T
• D0023550PF	2.36	0.03	PS•(470,525)DCM	Fl. Orange				10.0	G
• D0034000PF	3.40		PS•(480,520)XC estapor®	Fl. Pink				1.0	T
• D0034001PF	3.40		PS•(555,570)Y estapor®	Fl. Pink				1.0	T
• D0034002PF	3.40		PS•(broad,656)Z estapor®	Fl. Pink				1.0	T
• D0119501PF	11.95	0.108	P(S/20%DVB)•(502,513)	§ Fl. Yel-Grn				10.0	X1
• D0150000PF	15.00	0.49	P(S/2%DVB)•(500,515)	§ Fl. Yellow				0.2	U
• D0150001PF	15.00	0.49	P(S/2%DVB)•(530,560)	§ Fl. Orange				0.2	U
• D0150002PF	15.00	0.49	P(S/2%DVB)•(575,600)	§ Fl. Red				0.2	U
• D0150003PF	15.00	0.49	P(S/2%DVB)•(650,660)	§ Fl. Blue				0.2	U
• D4000000PF	400.00	150-1500	PP powder {Dry}•(350,615	Fl. White				100.0	X1
Carboxylate Functionalized									
D0001001CF	0.10	0.01	P(S/A/V-COOH)•(660,690)	Fl. Blue	COOH/1	277	34	9.6	D
D0001501CF	0.15		S/A/V-COOH•Rhodamine B	Fl. Pink	COOH/1			1.0	T
• D0001852CF	0.19		P(S/V-COOH)•(480,520)XC	Fl. Yel-Grn	COOH/1	516	10	1.0	T
• D0001853CF	0.19	0.01	P(S/V-COOH)•(555,570)Y e	Fl. Pink	COOH/1	516	10	1.0	T
D0002890CF	0.29	0.01	P(S/V-COOH)•(480,520)XC	Fl. Yel-Grn	COOH/1	430	8	1.0	T
• D0003000CF	0.30		P(S/V-COOH)•(broad,656)Z	Fl. Pink	COOH/1			1.0	T
• D0003280CF	0.33	0.02	P(S/V-COOH)•(555,570)Y e	Fl. Pink	COOH/1			1.0	T
• D0004170CF	0.42	0.01	P(S/V-COOH)•(555,570)Rhoi	Fl. Pink	COOH/1			1.0	T
• D0004190CF	0.42		P(S/V-COOH)•(660,690)	Fl. Blue	COOH/1	110	20	10.0	E
• D0008570CF	0.86		P(S/V-COOH)•(635,655)	§ Fl. Blue	COOH/1	9	122	10.0	E
• D0008990CF	0.90	0.02	P(S/V-COOH)•(397,409)U e	Fl. White	COOH/1	10	105	8.3	T
• D0008991CF	0.90	0.02	P(S/V-COOH)•(555,570)Y e	Fl. Pink	COOH/1	7	161	7.6	T
D0008992CF	0.90	0.02	P(S/V-COOH)•(460,480)X e	Fl. Yellow	COOH/1	3	348	1.0	T
• D0008993CF	0.90	0.02	P(S/V-COOH)•(480,520)XC	Fl. Yel-Grn	COOH/1	3	348	1.0	T
• D0009310CF	0.93		P(S/V-COOH)•(660,690)	Fl. Blue	COOH/1	110	9	10.0	E
• D0012120CF	1.21	0.02	P(S/V-COOH)•(555,570)Rhoi	Fl. Pink	COOH/1			1.0	T
Other Surface Functionalized									
• D0003400ZF	0.34		P(S/acrylamide)+hydrazin	§ Fl. Yellow	NHNH2			8.0	E
D0039500PF	3.95	0.22	P(S/R-OH)•(397,409)U est	Fl. White	CH2-OH			1.0	T

• Immediate Stock Availability

†See Legend and Price Schedule

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Abbreviation Legend

11/13/97

Polymer or Abbreviation	Meaning
A	Acrylic polymer, unspecified
AA	Acrylic Acid
Ar	Aromatic (benzene-ring linked)
B	Butadiene
BMA	ButylMethAcrylate
BrS	BromoStyrene
CH ₂ -Cl	Chloromethyl group
CH ₂ -OH	Alcohol (hydroxyl group)
CHO	Aldehyde group
CONH ₂	Amide group or monomer
COOH	Carboxylic Acid Group
COOH/1	COOH/Type 1: S/AA copolymer
COOH/2	COOH/Type 2: S/MA copolymer
COOH/4	COOH/Type 4: Type 1 + spacer arm
COOH/5	COOH/Type 5: copolymer with spacer arm ~10Å
DVB	DiVinylBenzene
EDA	EthyleneDiAmine
EDMA	EthyleneDiMethAcrylate
EMA	EthylMethAcrylate
Fl.	Fluorescent
GMA	GlycidylMethAcrylate
MA	Maleic Anhydride
MAA	MethAcrylic Acid
Mag.	Magnetite - Fe ₃ O ₄
MMA	MethylMethAcrylate
MS	MethylStyrene
NHNH ₂	Hydrazide group
P()	Poly(copolymer)
PMMA	PolyMethylMethAcrylate
pMS	paraMethylStyrene
PS	PolyStyrene
PVAc	PolyVinylAcetate
PVT	PolyVinylToluene
R	Aliphatic
Rsch	Research material
S	Styrene
SO ₃	Sulfonic acid groups
Std	Standard Material - replicate lots available.
V	Vinyl, unspecified polymerizable group
VBC	VinylBenzylChloride
VdCl	Vinylidene Chloride
VT	VinylToluene
XL	Cross-linked
___%	percentage of magnetite or monomer used
§	Anti microbial present (Sodium Azide or Merthiolate)
•	Before Stock no.= Immediate availability