Annexos

ANNEX 1

Domain bacteria

- ↳ Phylum Nitrospira
  - ↳ Class Nitrospira
    - ↳ Order Nitrospirales
      - ↳ Family Nitrospiraceae
        - ↳ Genus Nitrospira
          - ↳ ***Nitrospira moscoviensis***
          - ↳ ***Nitrospira marina***
          - ↳ ***Nitrospira sp.***
        - ↳ Genus Thermodesulfovibrio
          - ↳ *Nitrospira sp.*

- ↳ Phylum Proteobacteria
  - ↳ Class Alphaproteobacteria
    - ↳ Order Caulobacterales
      - ↳ Family Caulobacteraceae
        - ↳ Genus Brevundimonas
          - ↳ *Nitrobacter iranicum*
    - ↳ Order Rhizobiales
      - ↳ Family Bradyrhizobiaceae
        - ↳ Genus nitrobacter
          - ↳ ***Nitrobacter winogradsky***
          - ↳ ***Nitrobacter hamburguensi***

Cluster 6a192	NEU	Nmo 218	NmV	Nsm 156	NSMR 34	NSMR 76	Nso 1225	Nso 190	Nsv 443			

	Cluster 6a192	NEU	Nmo 218	NmV	Nsm 156	NSMR 34	NSMR 76	Nso 1225	Nso 190	Nsv 443			
↳ ***Nitrobacter hamburguensi***													
↳ ***Nitrobacter alkalicus***													
↳ ***Nitrobacter sp.***								+					
↳ Unclassified Bradyrhizobiaceae													
↳ *Nitrobacter sp.*													
↳ Class Betaproteobacteria													
↳ Order Nitrosomonadales													
↳ Family Nitrosomonadaceae													
↳ Genus Nitrosomonas													
↳ ***Nitrosomonas eutropha***		+			+			+	+				
↳ ***Nitrosomonas europaea***		+			+			+	+				
↳ ***Nitrosomonas halophila***		+						+	+				
↳ ***Nitrosomonas nitrosa***								+	+				
↳ ***Nitrosomonas communis***								+	+				
↳ ***Nitrosomonas sp.***	+	+		+	+			+	+				
↳ ***Nitrosococcus mobilis***				+					+				
↳ Genus Nitrosospira													
↳ ***Nitrosomonas marina***								+	+				
↳ ***Nitrosomonas cryotolerans***								+	+				
↳ ***Nitrosomonas ureae***			+					+					
↳ ***Nitrosomonas aestuarii***								+					
↳ ***Nitrosomonas sp***			+					+	+	+			
↳ ***Nitrosospira briensis***								+	+	+			
↳ ***Nitrosospira multiformis***						+		+	+	+			
↳ ***Nitrosospira tenuis***						+		+	+	+			
↳ ***Nitrosospira sp.***						+		+	+	+			
↳ ***Nitrosovibrio tenuis***								+	+	+			
↳ ***Nitrosovibrio sp.***								+	+				

	Cluster 6a192	NEU	Nmo 218	NmV	Nsm 156	NSMR 34	NSMR 76	Nso 1225	Nso 190	Nsv 443				
↳ Unclassified Nitrosomonadaceae								+						
↳ ***Nitrosomonas comunis***	+		+					+						
↳ ***Nitrosomonas oligotropha***								+						
↳ ***Nitrosomonas aestuari***			+					+						
↳ ***Nitrosomonas ureae***								+	+					
↳ ***Nitrosomonasmarina***			+					+						
↳ ***Nitrosomonas sp.***														
↳ Unclassified betaproteobacteria			+											
↳ *Nitrosomonas eureae*								+						
↳ ***Nitrosomonas sp.***														
↳ Class Gammaproteobacteria														
↳ Order Chromatiales														
↳ Family Ectothiorhodospiraceae														
↳ Genus Nitrococcus				+										
↳ *Nitrococcus mobilis*														
↳ Unclassified ectothiorhodospiraceae														
↳ *Nitrosococcus oceanis*														
↳ Unclassified Chromatiales														
↳ *Nitrosococcus oceanis*														
↳ *Nitrosococcus halophilus*														
↳ Unclassified Gammaproteobacteria														
↳ *Nitrsococcus oceani*														
↳ *Nitrosococcus halophilus*														
↳ Class Deltaproteobacteria														
↳ Order Desulfobacterales														
↳ Family Nitrospinaceae														
↳ Genus Nitrospina														
↳ *Nitrospina gracilis*				+										

ANNEX 2

The hybridization buffer composition is:

Component	Volume to prepare 2 mL (microcentrifuge tub)	Final concentration
5M NaCl (autoclaved)	360 µL	900mM
1M Tris/HCl (autoclaved)	40 µL	20mM
10% SDS (not autoclaved) placed in the lid of the tube	2 µL	0.01%
Formamide	0- 1600 µL [1]	0-80 % [1]
MilliQ water	up to 2 mL	-

[1] Formamide concentration depends on the probe used.

Probes targeting ammonia-oxidizing bacteria (AOB) and formamide (FA) concentration used in FISH:

Short name	**Specificity**	**FA (%)**	**Reference**
Cluster 6a192 [3]	Nitrosomonas oligothropha lineage (Cluster 6a)	35	Adamczyk et al. (2003)
NEU [3]	Most halophilic and halotolerant Nitrsomonas spp.	40	Wagner et al. (1995)
Nmo218	Nitrosomonas oligothropha-lineage	35	Gieseke et al. (2001)
NmV (Ncmob)	Nitrosococcus mobilis ("Nitrosomonas") lineage	35	Juretschko et al. (1998)
Nscoc1248	Nitrosococcus oceani, Nitrosococcus halophilus	[1]	Juretschko (2000)
Nscoc65	Nitrosococcus oceani, Nitrosococcus halophilus	[1]	Juretschko (2000)
Nscry1004	Nitrosomonas cryotolerans	[1]	Juretschko (2000)
Nse1472	Nitrosomonas europea, N. halophila, N. eutropha, Kraftisried-Isolat Nm103	50	Juretschko et al. (1998)
Nsm156	Nitrosomonas spp., Nitrosococcus mobilis	5	Mobarry et al. (1996)
NSMR34	Nitrosospira tenius-like ammonia-oxidizing bacteria	20	Burrell et al. (2001)
NSMR76	Nitrosomonas marina-like ammonia-oxidizing bacteria	20	Burrell et al. (2001)
Nso1225	Betaproteobacterial ammonia-oxidizing bacteria	35	Mobarry et al. (1996)
Nso190	Betaproteobacterial ammonia-oxidizing bacteria	55[2]	Mobarry et al. (1996)
Nsv443	Nitrosospira spp.	30	Mobarry et al. (1996)
NmII	Nitrosomonas communis-lineage	25	Pommerening-Röser et a. (1996)
NmIV	Nitrosomonas cryotolerans-lineage	-	Pommerening-Röser et a. (1996)
NOLI191	Some Nitrosomonas oligotropha-lineage	30	Rath (1996)

(1) Not published yet.

(2) Re-optimized at 35 %

(3) It should be used together with the competitive probe.

ANNEX 3

Probe aliquotes

Stock solution: 1 mg mL^{-1}

Working solution: 1 µg mL^{-1}

Store in 2 ml aliquots at –20°C (wrap tube in aluminum foil)

ANNEX 4 I 5

The washing buffer composition is:

Component	Volume to prepare 50 mL (falcon tub)	Final concentration
5M NaCl (autoclaved)	0-9 mL(1)	0-900mM(1)
0.5M EDTA (autoclaved)	0-500 µL(2)	0-5 mM(2)
1M Tris/HCl (autoclaved)	1 mL	20mM
MilliQ water	up to 50 mL	-
10% SDS (not autoclaved)	50 µL	0.01%

(1) NaCl concentration and (2) EDTA concentration depend on formamide concentration in the hybridization buffer. See table at the annexe.

Formamide, NaCl and EDTA concentrations in the hybridization and washing buffers:

In hybridization buffer		In washing buffer			
Final conc. Formamide (%)	Formamide (µL)(1)	Final conc. NaCl (mM)	Final conc. EDTA (mM)(2)	5M NaCl (µL)(3)	0.5M EDTA (µL)(3)
0	0	900	-	9000	-
5	100	630	-	6300	-
10	200	450	-	4500	-
15	300	318	-	3180	-
20	400	215	5	2150	500
25	500	149	5	1490	500
30	600	102	5	1020	500

35	700	70	5	700	500
40	800	46	5	460	500
45	900	30	5	300	500
50	1000	18	5	180	500
55	1100	10	5	100	500
60	1200	4	5	40	500
65	1300	- (4)	5	- (4)	500
70	1400	- (4)	3.5	- (4)	350
75	1500	- (4)	2.5	- (4)	250
80	1600	- (4)	1.75	- (4)	175

(1) To prepare 2 mL of hybridization buffer.

(2) EDTA is added at high stringencies to avoid influence of dications.

(3) To prepare 50 mL of washing buffer.

(4) Enough Na^+ in EDTA.

ANNEX 6

FITXA DE LES SONDES UTILITZADES (extret de: Probebase. Department of Microbial Ecology. University of Vienna)

EUB338 (Bact338) (bacterial)	Tested for in situ hybridization.
Accession no.	pB-00159
Name (Alm et al., 1996)*	S-D-Bact-0338-a-A-18
Specificity	most Bacteria **Check specificity using the ARB difference alignment*** **Check specificity using Probe Match at RDP II**
Probe category	**higher taxonomic levels**
Target molecule	16S rRNA
Position*	338 - 355
Sequence*	5'- GCT GCC TCC CGT AGG AGT -3'
Length [nt]	18
G+C content [%]	66,7
Tm [°C]*	55
delta Gs [kcal/mol]*	ΔG_1: -24,86; ΔG_2: -1,59; ΔG_{12}: -23,22
MW [g/mol]	5487
Formamide [%]*	0-50
Reference	Amann R. I., Binder B. J., Olson R. J., Chisholm S. W., Devereux R. and Stahl D. A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56: 1919-1925.

NEU	Tested for in situ hybridization.
Accession no.	pB-00382
Name (Alm et al., 1996)*	S-*-Nsm-0651-a-A-18
Specificity	Most halophilic and halotolerant Nitrosomonas spp. **Check specificity using Probe Match at RDP II**
Probe category	**bacteria of relevance in wastewater treatment** **ammonia-oxidizing bacteria**
Target molecule	16S rRNA
Position*	653 - 670
Sequence*	5'- CCC CTC TGC TGC ACT CTA -3'
Competitor	5'- TTC CAT CCC CCT CTG CCG -3'
Length [nt]	18
G+C content [%]	61,1
Tm [°C]*	53
delta Gs [kcal/mol]*	ΔG_1: -23,94; ΔG_2: 2,4; ΔG_{12}: -23,92
MW [g/mol]	5346
Formamide [%]*	40
Reference	Wagner M., Rath G., Amann R., Koops H.-P. and Schleifer K.-H. (1995). In situ identification of ammonia-oxidizing bacteria. Syst. Appl. Microbiol. 18: 251-264.

Nso190	Tested for in situ hybridization.
Accession no.	pB-00249
Name (Alm et al., 1996)*	S-F-bAOB-0189-a-A-19
Specificity	Betaproteobacterial ammonia-oxidizing bacteria **Check specificity using the ARB difference alignment*** **Check specificity using Probe Match at RDP II**
Probe category	**bacteria of relevance in wastewater treatment** **ammonia-oxidizing bacteria**
Target molecule	16S rRNA
Position*	189 - 207
Sequence*	`5'- CGA TCC CCT GCT TTT CTC C -3'`
Length [nt]	19
G+C content [%]	57,9
Tm [°C]*	53
delta Gs [kcal/mol]*	ΔG_1: -24,35; ΔG_2: 2,51; ΔG_{12}: -24,33
MW [g/mol]	5641
Formamide [%]*	55
Reference	Mobarry B. K., Wagner M., Urbain V., Rittmann B. E. and Stahl D. A. (1996). Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl. Environ. Microbiol. 62: 2156-2162

Nmo218	Tested for in situ hybridization.
Accession no.	pB-00957
Specificity	Nitrosomonas oligotropha-lineage **Check specificity using Probe Match at RDP II**
Probe category	**bacteria of relevance in wastewater treatment** **ammonia-oxidizing bacteria**
Target molecule	16S rRNA
Position*	218 - 235
Sequence*	`5'- CGG CCG CTC CAA AAG CAT -3'`
Length [nt]	18
G+C content [%]	61,1
Tm [°C]*	53
delta Gs [kcal/mol]*	ΔG_1: -20,43; ΔG_2: -0,99; ΔG_{12}: -19,31
MW [g/mol]	5451
Formamide [%]*	35
Reference	Gieseke A., Purkhold U., Wagner M., Amann R. and Schramm A. (2001). Community structure and activity dynamics of nitrifying bacteria in a phosphate-removing biofilm. Appl. Environ. Microbiol. 67: 1351-1362.

Nsv443	Tested for in situ hybridization.
Accession no.	pB-00250
Specificity	Nitrosospira spp. **Check specificity using the ARB difference alignment*** **Check specificity using Probe Match at RDP II**
Probe category	**bacteria of relevance in wastewater treatment** **ammonia-oxidizing bacteria**
Target molecule	16S rRNA
Position*	444 - 462
Sequence*	5'- CCG TGA CCG TTT CGT TCC G -3'
Length [nt]	19
G+C content [%]	63,2
Tm [°C]*	55
delta Gs [kcal/mol]*	ΔG_1: -23,15; ΔG_2: 0,5; ΔG_{12}: -22,92
MW [g/mol]	5743
Formamide [%]*	30
Reference	Mobarry B. K., Wagner M., Urbain V., Rittmann B. E. and Stahl D. A. (1996). Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl. Environ. Microbiol. 62: 2156-2162.

NIT3	Tested for in situ hybridization.
Accession no.	pB-00241
Name (Alm et al., 1996)*	S-G-Nbac-1035-a-A-18
Specificity	Nitrobacter spp. **Check specificity using the ARB difference alignment*** **Check specificity using Probe Match at RDP II**
Probe category	**bacteria of relevance in wastewater treatment** **nitrite-oxidizing bacteria**
Target molecule	16S rRNA
Position*	1035 - 1052
Sequence*	5'- CCT GTG CTC CAT GCT CCG -3'
Competitor	5'- CCT GTG CTC CAG GCT CCG -3'
Length [nt]	18
G+C content [%]	66,7
Tm [°C]*	55
delta Gs [kcal/mol]*	ΔG_1: -24,02; ΔG_2: -0,21; ΔG_{12}: -23,47
MW [g/mol]	5400
Formamide [%]*	40
Reference	Wagner M., Rath G., Koops H.P., Flood J. and Amann R. (1996). In situ analysis of nitrifying bacteria in sewage treatment plants. Wat Sci Techn 34: 237-244

ANNEX 7

PROGRAMA PER A DETERMINAR EL THRESHOLD

```
% Programa principal per a definir el threshold utilitzant les fotos Control

% Abans s'ha de crear el fitxer ".txt" que conte el nom de totes les
% imatges. Aquest fitxer estara grabat dins la carpeta on hi ha les fotos

clear all
close all
tic
ficheros=textread('C:\Documents and
settings\Irene\ijubany_FISH\060726\Mostra6_NEU_Control\llista_fotos.txt','%s'); %
Fitxer amb el nom de totes les fotos que volguem quantificar
numero=length(ficheros);
directorio='C:\Documents and
settings\Irene\ijubany_FISH\060726\Mostra6_NEU_Control\'; % Directori on hi ha les
fotos

for i=1:numero/2

   %Fotos blaves (sonda general)
   fichero=strcat(directorio,ficheros(i*2-1)); %construeix cadena nom fitxer
   fichero= char(fichero); %transforma cadena a caracters
   [Xb, mapb]=imread(fichero);    % Llegeix la imatge
   maxXb=max(max(Xb));

   % Crea els histogrames de les imatges del canal blau (en logaritimic o
   % en normal)
   figure('position', [50 75 900 600])
   figure(i);
   subplot(1,2,1)
   [counts,x]=imhist(Xb);
%     semilogy(x,counts)
   plot(x,counts)
   axis([0 100 0 100])
   title('Blau');

   % Busca el valor d'intensitat en el qual hi ha inclosos el 99.99% dels
   % punts
   blau=0;
   for npuntsb=5:200
      if blau<99.99
         blau=sum(counts(1:npuntsb))/sum(counts)*100;
         blau2(i)=blau;
         intensitat_blau(i)=npuntsb;
      end
   end
```

```
   % Fotos vermelles (sonda especifica)
   fichero=strcat(directorio,ficheros(i*2)); %construeix cadena nom fitxer
   fichero= char(fichero); %transforma cadena a caracters
   [Xv, mapv]=imread(fichero);     % Llegeix la imatge
   maxXv=max(max(Xv));

   % Crea els histogrames de les imatges del canal vermell (en logaritmic
   % o en normal)

   [counts,x]=imhist(Xv);
   subplot (1,2,2);
   plot(x,counts)
%     semilogy(x,counts)
   axis([0 100 0 100])
   title('Vermell');

   % Busca el valor d'intensitat en el qual hi ha inclosos el 99.99% dels
   % punts
   vermell=0;
   for npuntsv=5:200
      if vermell<99.99
         vermell=sum(counts(1:npuntsv))/sum(counts)*100;
         vermell2(i)=vermell;
         intensitat_vermell(i)=npuntsv;
      end
   end

end;

% Mosta en pantalla la intensitat threshold trobada per a cada foto i el
% promig dels thresholds. Ho mostra per a les imatges blaves i les
% vermelles

blau2
intensitat_blau
promig_blau=sum(intensitat_blau)/(numero/2)

vermell2
intensitat_vermell
promig_vermell=sum(intensitat_vermell)/(numero/2)

toc
```

PROGRAMA PRINCIPAL PER A FER LA QUANTIFICACIO

Programa principal per a quantificar les imatges del FISH i del seu error

```
% Abans s'ha de crear el fitxer ".txt" que conte el nom de totes les
% imatges. Aquest fitxer estara grabat dins la carpeta on hi ha les fotos

clear all
close all
tic
ficheros=textread('C:\Documents and
settings\Irene\ijubany_FISH\060726\Mostra6_NEU_totes\llista_fotos.txt','%s'); %
Fitxer amb el nom de totes les fotos que volguem quantificar
display('numero de fotos')
numero=length(ficheros)
directorio='C:\Documents and
settings\Irene\ijubany_FISH\060726\Mostra6_NEU_totes\'; % Directori on hi ha les
fotos

% Threshold que hem establert anteriorment
threshold_b=25;
threshold_v=20;
interval=5; % interval d'intensitat per a trobar l'error de la quantificacio

% Quantificacio amb els thresholds corresponents
thresh_b=threshold_b;
thresh_v=threshold_v;
display('valor mig')
quantificaciofish_patro2

% Quantificacio amb els thresholds modificats per a trobar el valor minim
close all
thresh_b=threshold_b-interval;
thresh_v=threshold_v+interval;
display('valor minim')
quantificaciofish_patro2

% Quantificacio amb els thresholds modificats per a trobar el valor maxim
close all
thresh_b=threshold_b+interval;
thresh_v=threshold_v-interval;
display('valor maxim')
quantificaciofish_patro2
```

SUBRUTINA PER A FER LA QUANTIFIACIO, QUE ÉS CRIDADA DES DEL PROGRAMA PRINCIPAL

```
Per a fotos en les quals hi ha punts vermells que en realitat no son
% biomassa i es volen treure informaticament


% Calculo les imatges en blanc i negre pel canal vermell i sumo els punts
% blancs

for i=1:numero/2;

   %Fotos blaves (sonda general)
   fichero=strcat(directorio,ficheros(i*2-1)); %construeix cadena nom fitxer
   fichero= char(fichero); %transforma cadena a caracters
   [Xb, mapb]=imread(fichero);    % Llegeix la imatge
   Xblau_bw(:,:)=im2bw(Xb,thresh_b/256); %Transforma la imatge a BN
%    Xblau_bw(:,:,i)=Xblau_bwtmp | Xvermell_bw(:,:,i);
%       %Inclou els pixels vermells de la imatge complementaria (garanteix % < 100%)
   suma_blau(i)=sum(sum(Xblau_bw(:,:))); %Suma tots els punts BN

   % Fotos vermelles (sonda especifica)
   fichero=strcat(directorio,ficheros(i*2)); %construeix cadena nom fitxer
   fichero= char(fichero); %transforma cadena a caracters
   [Xv, mapv]=imread(fichero);     % Llegeix la imatge
   Xvermell_bwtmp=im2bw(Xv,thresh_v/256);  %Transforma la imatge a BM
   T=Xvermell_bwtmp | Xblau_bw(:,:); % operador logic OR
   Xvermell_bw(:,:)=Xvermell_bwtmp-T+Xblau_bw(:,:); % operacio per a treure els
punts vermells que no son biomassa
   suma_vermell(i)=sum(sum(Xvermell_bw(:,:)));   %Suma tots els punts BN

   quant=suma_vermell(i)/suma_blau(i);

   figure('position', [50 75 900 600])
   figure(i)
   subplot(2,3,1),    imshow(Xb)
   title(ficheros(i*2));
   subplot(2,3,2),    imshow(Xblau_bw(:,:))
   subplot(2,3,3),    [counts,x]=imhist(Xb); semilogy (x,counts), hold on,
                  plot([thresh_b thresh_b],[1e2 1e4],'r')%, axis([0 256 0 50])
   subplot(2,3,4),    imshow(Xv)
   subplot(2,3,5),    imshow(Xvermell_bw(:,:))
   title(quant*100);
   subplot(2,3,6),    [counts,x]=imhist(Xv); semilogy (x,counts), hold on,
                   plot([thresh_v thresh_v],[1e2 1e4],'r') %, axis([0 256 0 50])

end;

suma_de_blau=sum(suma_blau)
suma_de_vermell=sum(suma_vermell)
```

```
quanti=suma_vermell./suma_blau.*100; % Calcula el % de vermell en blau
mitjana=mean(quanti)
desviacio=std(quanti)

quanti_total=sum(suma_vermell)/sum(suma_blau)*100

figure(100)
clf
plot(quanti)
hold on
plot([1:numero/2], mitjana*ones(1,numero/2),'c-')
plot([1:numero/2], quanti_total*ones(1,numero/2),'k-')

Xlabel('# foto')
Ylabel('% vermell sobre blau')

figure(101)
clf
plot(suma_blau,'b')
hold on
plot(suma_vermell,'r')
hold off

toc
```

ANNEX 8

Formamide

Product Number **F 9037**

Storage Temperature 2-8 □C

Product Description

Molecular Formula: CH3NO

Molecular Weight: 45.04

CAS Number: 75-12-7

Melting Point: 2.55 □C1

Boiling Point: 210.5 □C (760 torr; partial decomposition occurs into carbon monoxide and ammonia at atmospheric pressure starting at 180 □C)1

Molarity (neat liquid): 25.09 (based on density of 1.13 g/ml)

Synonyms: methanamide; carbamaldehyde

This product is designated as Molecular Biology grade and is suitable for use in nucleic acid hybridizations. It is deionized and packaged under argon in amber glass bottles.

Once the bottle is opened and the formamide is exposed to oxygen, this product will begin to oxidize to formic acid. Therefore, once opened, the bottle will need to be purged with nitrogen and stored frozen to prevent oxidation. Formamide is a reagent that is an ionizing solvent in
aqueous buffers. It is widely utilized in biochemistryand molecular biology, particularly in nucleic acids research.

Procedures have been reported for the use of formamide in DNA sequencing and in polyacrylamide sequencing gels, which helps to eliminate secondary structure in nucleic acids and thus compressions in the gel data. Other protocols incorporate formamide for RNA denaturation, Northern blot hybridization and stripping, ribonuclease protection assays, RNA storage, and Southern hybridization.2 The use of formamide for the quantitation of mRNAs in hepatocytes solubilized in guanidium thiocyanate has been

reported.3 Protocols have been described for the use of ormamide in DNA isolation from cultured bacteriophage □and cultured mammalian cells.2

Formamide may be included at levels up to 50% in a streptavidin-induced electrophoretic mobility shift procedure for the isolation of single-stranded DNA from PCR products.4 Formamide is also utilized in such large scale applications as the manufacture of formic esters, the production of hydrocyannic acid by catalytic dehydration, and as a softener for paper.1 A study of the use of formamide to recover estradiol degradation products in a transdermal drug delivery formulation has been published.5 A protocol that incorporates formamide into the analysis of □-blocker compounds by nonaqueous capillary electrophoresis coupled to electrospray ionization mass spectrometry has been described.6

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

This product is miscible in water (formamide:water, 50% v/v), yielding a clear, colorless solution.