

Feb 01, 2023

🌐 Preparation of Buffers for PhageFISH protocol

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📁 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.dm6gpjop8gzp/v1

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OPEN ACCESS



DOI: dx.doi.org/10.17504/protocols.io.dm6gpjop8gzp/v1

Protocol Citation: Line Jensen Ostenfeld, Saria Otani 2023. Preparation of Buffers for PhageFISH protocol. [protocols.io](https://dx.doi.org/10.17504/protocols.io.dm6gpjop8gzp/v1)
<https://dx.doi.org/10.17504/protocols.io.dm6gpjop8gzp/v1>

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Protocol status: Working

We use this protocol and it's working

Created: January 27, 2023

Last Modified: February 01, 2023

Protocol Integer ID: 75961

Keywords: Permeabilisation buffer, rRNA hybridisation buffer, rRNA hybridisation wash buffer, rRNA CARD buffer, Gene hybridisation buffer, Gene hybridisation wash buffer

Funders Acknowledgements:

NovoNordisk

Grant ID: NNF16OC0021856

Abstract

This protocol details about preparation of various buffers for PhageFISH protocol.

Attachments



[627-1301.docx](#)

32KB

Materials

Materials and Reagents




- PBS [pH 7.5] (10 x)
- Tris-HCl [pH 8.0](1 M)
- EDTA (0.5)
- Water
- Lysozyme
- Dextran sulphate
- NaCl (5 M)
- Tris-HCl [pH 8.0] (1 M)
- Nucleic acid blocking reagent (10%)
- Sheared salmon sperm (10 mg/ml)
- Yeast RNA (10 mg/ml)
- Formamide (100%)
- SDS (20%)
- SSC (20 x)
- 0.22µm syringe filter
- 50 ml falcon tube

Permeabilisation buffer

1 50 ml:

A	B
PBS [pH 7.5] (10 x)	5 ml
Tris-HCl [pH 8.0] (1 M)	5 ml
EDTA (0.5)	5 ml
Water	35 ml
Lysozyme	

Permeabilisation mix: Mix PBS, Tris-HCl, EDTA, and water.


2 High conc. lysozyme buffer: Dissolve lysozyme in appropriate buffer volume ( 50 mg lysozyme to  10 mL buffer). It may be necessary to heat the solution to  37 °C .

3 Dilute lysozyme buffer into large buffer volume by adding 1 part lysozyme buffer to 9 parts permeabilisation mix.

4 Final concentration:

A	B
PBS	1 x
Tris-HCl	0.1 M
EDTA	0.05 M
Lysozyme	0.5 mg/ml

Note


- Permeabilisation buffer should not be stored.
- Prepare in aliquots of  1 mL .


rRNA hybridisation buffer



5 40 ml:

A	B
Dextran sulphate	4 g

A	B
NaCl (5 M)	7.2 ml
Tris-HCl [pH 8.0] (1 M)	0.8 ml
Water	4 ml
Nucleic acid blocking reagent (10%)	4 ml
Sheared salmon sperm (10 mg/ml)	1 ml
Yeast RNA (10 mg/ml)	1 ml
Formamide (100%)	17.5 ml
SDS (20%)	40 µl

Mix dextran sulphate, NaCl, Tris-HCl, and water in a falcon tube and vortex or shake thoroughly to disperse dextran sulphate. Heat solution in waterbath at  37-48 °C and vortex until dextran sulphate is completely dissolved.

6 Cool the solution down to  Room temperature .



7 Add nucleic acid blocking agent, sheared salmon sperm, yeast RNA, formamide, and SDS. Adjust volume with water to reach  40 mL if necessary. 


8 Vortex to mix. 

9 Spin down solution briefly and filter through 0.22µm syringe filter.

10 Final concentration:

A	B
Dextran sulphate	10%
NaCl	0.9 M
Tris-HCl	20 mM
Nucleic acid blocking reagent	1%
Sheared salmon sperm	0.25 mg/ml
Yeast RNA	0.25 mg/ml
Formamide	35%
SDS	0.02%

11 Store in aliquots at  -20 °C . Reheat to  37 °C before use to redissolve precipitate.

12 Prepare several in aliquots of  900 µL .


rRNA hybridisation wash buffer

13 50 ml:

A	B
*NaCl (5 M)	700 µl
*EDTA [pH 8.0] (0.5 M)	500 µl
Tris-HCl (1 M)	1 ml
Water	up to 50 ml
SDS (20%)	25 µl

Mix *NaCl, *EDTA, and Tris-HCl in 50 ml falcon tube.

Note


* **NOTE:** Na⁺ concentrations depend on the amount of formamide used in the hybridisation buffer. The formamide concentration is calculated based on probe properties to achieve a hybridisation temperature of  42-50 °C .

14 Add water up the 50 ml mark.

15 Add SDS.

16 Final concentrations:

A	B
NaCl	70 mM
EDTA	5 mM
Tris-HCl	20 mM
SDS	0.01%

- 17 The formamide (FA) concentrations and the corresponding Na⁺ ions concentrations when washing at  48 °C are as follows:

A	B
0% FA	900 mM Na ⁺
5% FA	636 mM Na ⁺
10% FA	450 mM Na ⁺
15% FA	318 mM Na ⁺
20% FA	225 mM Na ⁺
25% FA	159 mM Na ⁺
30% FA	112 mM Na ⁺
35% FA	80 mM Na ⁺
40% FA	56 mM Na ⁺
45% FA	40 mM Na ⁺
50% FA	28 mM Na ⁺
55% FA	20 mM Na ⁺
60% FA	14 mM Na ⁺

- 18 Prepare in aliquots of 50.

Note


Prepare at least two aliquots per cycle.

rRNA CARD buffer

- 19 40 ml:



A	B
Dextran sulphate	4 g
PBS [pH 7.4] (10 x)	4 ml
NaCl (5 M)	16 ml
Water	up to 40 ml
Nucleic acid blocking reagent (10%)	400 µl

Mix dextran sulphate, PBS, and NaCl. Add water up to 40 ml. vortex thoroughly to disperse dextran sulphate. Heat solution in waterbath at  37-48 °C and vortex until dextran sulphate is completely dissolved.

20 Allow solution to cool down to room temperature and add nucleic acid blocking reagent.

21 Vortex to mix.


22 Spin down briefly.

23 Filter through 0.22 µm syringe filter.

24 Final concentration:

A	B
PBS	1x
Dextran sulphate	10%
Nucleic acid blocking reagent	0.10%
NaCl	2 M

25 Store in aliquots at  4 °C . Reheat to  37 °C before use to redissolve precipitate.


26 Prepare in aliquot of  3 mL .


Gene hybridisation buffer


27 40 ml:

A	B
Dextran sulphate	4 g
SSC (20 x)	10 ml
EDTA [pH 8.0] (0.5 M)	1.6 ml
Water	4.4 ml
Nucleic acid blocking reagent (10%)	4 ml
Sheared salmon sperm (10 mg/ml)	1 ml
Yeast RNA (10 mg/ml)	1 ml

A	B
Formamide (100%)	14 ml
SDS (20%)	200 μ l

Mix dextran sulphate, SSC, EDTA, and water in a falcon tube and vortex or shake thoroughly to disperse dextran sulphate. Heat solution in waterbath at  37-48 °C and vortex until dextran sulphate is completely dissolved.

28 Cool the solution down to  Room temperature .



29 Add nucleic acid blocking agent, sheared salmon sperm, yeast RNA, formamide, and SDS. 

30 Vortex to mix. 


31 Spin down solution briefly and filter through 0.22 μ m syringe filter.

32 Final concentration:

A	B
Formamide	35%
SSC	5x
Dextran sulphate	10%
SDS	0.10%
EDTA	20 mM
Nucleic acid blocking reagent	1%
Sheared salmon sperm	0.25 mg/ml
Yeast RNA	0.25 mg/ml

33 Store in aliquots at  -20 °C . Reheat to  42 °C before use to redissolve precipitate.

Gene hybridisation wash buffer I

34 50 ml: 

A	B
SSC (20 x)	5 ml

A	B
SDS	250 µl
Water	up to 50 ml

Mix SSC and water in a 50 ml falcon tube.

35 Add SDS.




36 Vortex to mix.



37 Final concentration:

A	B
SSC	2 x
SDS	0.1%

38 Store for 1-2 days at  42 °C .

Gene hybridisation wash buffer II

39 50 ml:



A	B
SSC (20 x)	250 µl
SDS	250 µl
Water	up to 50 ml

Mix SSC and water in a 50 ml falcon tube.

40 Add SDS.




41 Vortex to mix.



42 Final concentration:

SSC	0.1 x
SDS	0.10%


43 Store for 1-2 days at  42 °C .

Gene CARD amplification buffer

44 40 ml:

A	B
Dextran sulphate	8 g
PBS [pH 7.4] (10 x)	4 ml
NaCl (5 M)	16 ml
Water	15.6 ml
Nucleic acid blocking reagent (10%)	400 µl

Mix dextran sulphate, PBS, NaCl, and water.

45 Vortex or shake thoroughly to disperse dextran sulphate. Heat solution in waterbath at  37-48 °C and vortex until dextran sulphate is completely dissolved.

46 Allow solution to cool down to room temperature and add nucleic acid blocking reagent.



47 Vortex to mix.

48 Spin down briefly.

49 Filter through 0.22 µm syringe filter.

50 Final concentrations:

A	B
PBS	1x
Dextran sulphate	20%
Blocking reagent	0.10%
NaCl	2 M

51 Store in aliquots at  4 °C . Reheat to  37 °C before use to redissolve precipitate.

