Preparation of Buffers for PhageFISH protocol

In 1 collection

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We use this protocol and it's working

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ABSTRACT

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

ATTACHMENTS

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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

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

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
	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

	A	B
	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+

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

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 µm syringe filter.

32 Final concentration:

	A	B
	Formamide	35%

	A	B
	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
	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

	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

	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.