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## **EZ-Hybridization Solution**

### **Cat. No.: 01-889-1B (100ml)**

**Storage:** Room temperature  
For long term storage, store at 2-8°C

### **Product Description**

EZ-Hybridization Solution enables shorter hybridization times and decreases backgrounds. Therefore, low-copy RNA species on Northern blots and single-copy genes on Southern blots can be detected within 1-2 hours of hybridization using radioactively or non-radioactively labeled probes (compared to 12-24 hours with standard buffers).

### **cDNA Probes**

#### **1. General considerations**

- 1.1 EZ-Hybridization Solution can be used for Southern blot, Northern blot, or colony hybridizations. The only difference between the protocols is the incubation temperature.  
*Note: Use 68°C for Northern; use 60°C for Southern*
- 1.2 The hybridization temperature used in the following protocols is suitable for hybridization of DNA probes of average G/C content (40%). The optimal temperature for probes of different G/C content must be determined empirically (Sambrook *et al.*, 1989)
- 1.3 The recommended final DNA probe concentration is 2-10ng/ml or 1-2x10<sup>6</sup> cpm/ml for Northern or Southern hybridizations. (Probe concentrations >10ng/ml will reduce the time needed for hybridization, but may increase background). For high target applications, e.g. colony hybridizations, lower probe concentration can be used.
- 1.4 Concentrated probe should not be added directly to the membrane because uneven concentrations may result in localized background.
- 1.5 EZ-Hybridization Solution allows shorter hybridization times, e.g. 1-2 hours. Nevertheless, overnight hybridization can still be performed.  
*Note: Long hybridization time, combined with high probe concentration, may lead to high background.*

#### **2. Hybridization with EZ-Hybridization Solution with radioactively labeled probes**

- 2.1 Warm the EZ-hybridization Solution at 68°C (60°C), and stir well to completely dissolve any precipitate.
- 2.2 Pre-hybridize membranes in a minimum of 0.1ml/cm<sup>2</sup> of EZ-Hybridization Solution with shaking at 68°C (60°C) for 30 minutes. The volume of solution must be sufficient to completely cover the membrane, or high backgrounds may result.
- 2.3 Denature the radioactively labeled DNA probe at 95-100°C for 2-5 minutes. Chill quickly on ice.
- 2.4 Add radiolabeled probe to a sufficient volume of fresh EZ-Hybridization Solution. Mix gently. For recommended final concentration, see above (1.3).
- 2.5 Replace the EZ-Hybridization Solution with the fresh solution containing the radiolabeled DNA probe. Remove all air bubbles from the container, and make sure the EZ-Hybridization Solution is evenly distributed over the entire blot.

- 2.6 Hybridize with continuous shaking at 68°C (60°C) for 1-2.5 hours. (For high target applications, shorter hybridization times can be used. For single-gene sequences, hybridization can be performed overnight).
- 2.7 Rinse the blot in a large amount of 2xSSC, 0.1% SDS 2-3 times at room temperature. Wash with at least 0.5ml/cm<sup>2</sup> of 2xSSC, 0.1% SDS for 30-40 minutes with continuous agitation; replace the wash solution twice.
- 2.8 Wash the blot in at least 0.5ml/cm<sup>2</sup> of 0.1xSSC, 0.1% SDS with continuous shaking at 65°C (65°) for 40 minutes with two changes of fresh solution. (For certain RNA probes, washing temperature above 65°C (up to 70°C) may be necessary in order to obtain the correct stringency).
- 2.9 Remove the blot with forceps and shake off excess wash solution.  
*Note: Do not blot-dry or allow the membrane to even partially dry. If the membrane is allowed to dry, subsequent removal of the probe from the membrane for re-probing may be difficult.*
- 2.10 For <sup>32</sup>P-labeled probes, immediately cover the blot with plastic wrap. Mount on Whatman paper (3MM). Wrap again with plastic wrap. For <sup>35</sup>S-labeled probes, autoradiograph dried membranes at room temperature without plastic wrap.
- 2.11 Expose to x-ray film at -70°C with two intensifying screens.
- 2.12 If the membrane is to be re-probed, incubate the blot in sterile H<sub>2</sub>O containing 0.5% SDS as outlined below.
  - 2.12.1 Heat the sterile H<sub>2</sub>O/0.5% SDS solution to 90-100°C.
  - 2.12.2 Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
  - 2.12.3 Incubate for 10 minutes, shaking frequently.
  - 2.12.4 Allow the solution to cool for 10 minutes before removing the blot.
  - 2.12.5 Remove the blot and air-dry until it is dry enough to be slipped into plastic bag. The membrane can be stored at -20°C until needed.

### 3. Hybridization using EZ-Hybridization Solution with non-radioactively labeled probes

- 3.1 Warm the EZ-Hybridization Solution at 68°C (60°C) and stir until completely dissolved any precipitate.
- 3.2 Pre-hybridize membranes in a minimum of 0.1ml/cm<sup>2</sup> of EZ-Hybridization Solution with continuous shaking at 68°C (60°C) for 30 minutes. The volume of solution must be sufficient to completely cover the membrane, or high backgrounds may result.
- 3.3 Denature the radioactively labeled DNA probe at 95-100°C for 2-5 minutes. Chill quickly on ice.
- 3.4 Add non-radiolabeled probe to a sufficient volume of fresh EZ-Hybridization Solution. Mix gently. For recommended final probe concentrations, see above (1.3).
- 3.5 Replace the EZ-Hybridization Solution with the fresh solution containing the non-radiolabeled DNA probe. Remove all air bubbles from the container, and make sure the EZ-Hybridization Solution is evenly distributed over the entire blot.
- 3.6 Hybridize with continuous shaking at 68°C (60°C) for 1-2.5 hours. (For high target applications, shorter hybridization times can be used. For single-gene sequences, hybridization can be performed overnight).
- 3.7 Wash the membranes at room temperature twice, 15 minutes each time, with at least 0.5ml/cm<sup>2</sup> of 2xSSC, 0.1% SDS.
- 3.8 Wash the membrane at 68°C (60°C) twice, 15 minutes each time, with at least 0.5ml/cm<sup>2</sup> of 1-0.1xSSC, 0.1% SDS, with the continuous agitation.  
*Note: These washing conditions may be too stringent for probes which are not completely homologous to the target. If this is the case, lower the temperature to 50°C.*
- 3.9 Remove the blot with forceps and shake off excess wash solution. Blots can then be used directly for chemiluminescent detection (EZ-ECL, Cat. No. 20-500-120) of hybridized DNA, or stored air-dried for later detection using colorimetric detection systems.

## Oligonucleotide Probes

### 1. General considerations

- 1.1 EZ-Hybridization Solution can be used with oligonucleotide probes in Northern blot or Southern blot hybridization.
- 1.2 The hybridization temperature used in the following protocols is suitable for hybridization of oligonucleotide probes of average G/C content (40%). The optimal temperature for probes of different G/C content must be determined empirically (1).
- 1.3 The recommended final oligonucleotide probe concentration is 10-50ng/ml or  $0.5-2 \times 10^7$  cpm/ml. Probe concentration of >50ng/ml will reduce the time needed for hybridization, but may increase background.
- 1.4 Concentrated probe should not be added directly to the membrane, because uneven concentrations may result in localized background.
- 1.5 EZ-Hybridization Solution allows shorter hybridization times, e.g. 30-60 minutes.

### 2. Hybridization with EZ-Hybridization Solution with radioactively labeled oligonucleotides

- 2.1 Warm the EZ-Hybridization Solution at 42°C.
- 2.2 Pre-hybridize membranes in a minimum of 0.1ml/cm<sup>2</sup> of EZ-Hybridization Solution with continuous shaking at 42°C for 30 minutes. The volume of solution must be sufficient to completely cover the membrane, or high background may result.
- 2.3 Add radiolabeled probe to a sufficient volume of fresh EZ-Hybridization Solution. Mix gently. For recommended final probe concentrations, see above (1.3).
- 2.4 Replace the EZ-Hybridization Solution with the fresh solution containing the radiolabeled oligonucleotide probe. Remove all air bubbles from the container and make sure the EZ-Hybridization Solution is evenly distributed over the entire blot.
- 2.5 Hybridize with continuous shaking at 42°C for 30-60 minutes. For high target applications, shorter hybridization times can be used. For single-gene sequences, hybridization can be performed overnight, but it can also lead to increased background.
- 2.6 Wash the membranes at room temperature, 20 minutes in 0.5ml/cm<sup>2</sup> of 5xSSC, 0.1% SDS.
- 2.7 Wash the membranes at 42°C, 15 minutes each time in 0.5ml/cm<sup>2</sup> of 1-0.1xSSC, 0.1% SDS.
- 2.8 Remove the blot with forceps and shake off excess wash solution.  
*Note: Do not blot-dry or allow the membrane to even partially dry. If the membrane is allowed to dry, subsequent removal of the probe from the membrane for re-probing may be difficult.*
- 2.9 For <sup>32</sup>p-labeled probes, immediately cover the blot with plastic wrap. Mount on Whatman paper (3MM). Wrap again with plastic wrap. For <sup>35</sup>s-labeled probes, autoradiograph dried membranes at room temperature without plastic wrap.
- 2.10 Expose to x-ray film at -70°C with two intensifying screens.
- 2.11 If the membrane is to be re-probed, incubate the blot in sterile H<sub>2</sub>O containing 0.5% SDS as outlined below.
  - 2.11.1 Heat the sterile H<sub>2</sub>O/0.5% SDS solution to 90-100°C.
  - 2.11.2 Remove plastic wrap from blot and immediately place in the heated solution. Make sure that exposure to air is minimal.
  - 2.11.3 Incubate for 10 minutes, shaking frequently.
  - 2.11.4 Allow the solution to cool for 10 minutes before removing the blot.
  - 2.11.5 Remove the blot and air-dry until it is dry enough to be slipped into a plastic bag. The membrane can be stored at -20°C until needed.

### 3. Hybridization with EZ-Hybridization Solution with non-radioactively labeled oligonucleotides

- 3.1 Warm the EZ-Hybridization Solution at 42°C.
- 3.2 Pre-hybridize membranes in a minimum of 0.1ml/cm<sup>2</sup> of EZ-Hybridization Solution with continuous shaking at 42°C for 30 minutes. The volume of solution must be sufficient to completely cover the membrane, or high backgrounds may result.
- 3.3 Add non-radiolabeled probe to a sufficient volume of fresh EZ-Hybridization Solution. Mix gently. For recommended final probe concentration, see above (1.3).
- 3.4 Replace the EZ-Hybridization Solution with the fresh solution containing the non-radiolabeled oligonucleotide probe. Remove the air bubbles from the container and make sure the EZ-Hybridization Solution is evenly distributed over the entire blot.
- 3.5 Hybridize with continuous shaking at 42°C for 30-60 minutes.
- 3.6 Wash the membranes at room temperature for 30 minutes with a minimum of 0.5ml/cm<sup>2</sup> of 5xSSC, 0.1% SDS, with continuous agitation. Replace the wash solution once.
- 3.7 Wash the membranes at 42°C for 30 minutes with a minimum of 0.5ml/cm<sup>2</sup> of 1-0.1xSSC, 0.1% SDS, with continuous agitation. Replace the wash solution once.
- 3.8 Remove the blot with forceps and shake off excess wash solution. Blots can then be used directly for chemiluminescent detection (EZ-ECL, Cat. No. 20-500-120) of hybridized DNA, or can be stored air-dried for later detection using colorimetric detection systems.

#### References:

- 1) Sambrook, J. Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY)
- 2) Wahl, G.M., Berger, S.L. & Kimmel, A.R. (1987) Molecular Hybridization of immobilized nucleic acids: Theoretical concepts and practical considerations. *Methods Enzymol.* **152**:399-407.

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