GeneDetect.com Ltd

North America:
1901 60th Place
Suite L8299
Bradenton, FL
USA 34203
Phone: 1-305-723-0628
Fax: 1-305-723-0628

Worldwide & Asia-Pacific:
209 Taylor Street, Suite 4
Blockhouse Bay
Auckland 0600
New Zealand
Phone: +64-9-353-1320
Fax: +64-9-353-1320

Website: www.GeneDetect.com
Email: Info@GeneDetect.com

Oligonucleotide Probe Information Sheet

Approx Mr of an unmodified oligonucleotide probe 300 X length (in base pairs)

Oligonucleotide probe conversion 1 OD (260) = 33 μg of unmodified oligonucleotide

Storage and stability.

Standard lyophilized oligonucleotides are stable for many years if stored frozen at -20°C.

When in solution however to prolong shelf life the oligonucleotide should be kept frozen at -20°C preferably in Tris-EDTA (10 mM, 1 mM) buffer at pH 7,5-8,0.

A slightly alkaline buffer prevents possible depurination of the oligonucleotide. In an acid buffer A and G bases are eliminated, leading to oligonucleotide damage. While TE buffer is the preferred solution for oligonucleotide storage it is not necessarily the best solution for oligonucleotide use. TE buffer may interfere with various enzyme dependent reactions so the required amount of oligonucleotide probe should be removed from the stock solution and diluted in nuclease free distilled water for labeling or other procedures.

While oligonucleotides are very stable they are prone to BACTERIAL attack. Use autoclaved tips and tubes.

Storage versus (stability) for GeneDetect® Oligonucleotide Probes.

Dissolved at 25°C (1 week to 3 months)
Lyophilized at 25°C (2 months to 1 year)
Dissolved (<<15μM) at –20°C (1 year to 3 years)
Dissolved (>15μM) at –20°C (3 years+)
Lyophilized at –20°C (indefinite)

5′ or 3′ labeled oligonucleotide probes should be kept away from air. Make sure the aliquot tube screw cap is on properly and that tubes are kept in properly closed ziplock bags should suffice.

Repeated freeze-thawing can dramatically lower the shelf life of an oligonucleotide.

Resuspending

Centrifuge the tube for a few seconds to ensure the oligonucleotide probe is fully collected at the bottom of the tube. Carefully open tube, add an appropriate amount of TE buffer or sterile nuclease free water, close, allow to rehydrate for 2 minutes. Mix the tube by hand for a minute before using a vortex on the solution for 15 seconds. Re-centrifuge the solution. Aliquot and/or store the oligonucleotide probe.

ALWAYS re-vortex the oligonucleotide probe solution before taking a sample to add to hybridization buffer. This is especially important when the oligonucleotide probe has been stored in solution for a prolonged period.

Recommended Probe concentrations to use in your hybridization experiment.

A number of factors determine the optimal concentration of probe to use in your hybridization experiments. These include the quality of the tissue and how it was fixed, the level of labeling of the probe and the sensitivity of the detection system used. We suggest that a probe dilution range be tested (see below). Freshly prepared tissue is always advantageous. For non-radioactive in situ hybridization we recommend using tyramide signal amplification (TSA) if possible as part of your detection protocol. TSA "all-in-one" kits are supplied by most major vendors and TSA has been shown to increase the sensitivity of non-radioactive in situ hybridization by up to 100Xs. By combining GreenStar*™ hyper-labeled probes with TSA detection it is possible to dramatically lower the concentration of probe required to detect your target mRNA which then reduces the likelihood of non-specific, background staining becoming a problem.

Determining the optimal Probe concentration for your non radioactive *in situ hybridization* experiment:

Dilute 5μg of GreenStar*™ labeled GeneDetect® probe in 500μl TE or sterile water as directed and prepare the following series of probe dilutions in hybridization buffer. If possible run the experiment on tissues known to express the target gene.

Dilution of probe in hybridization buffer	Final probe concentration (ng/ml)
1:10	(1000ng/ml)
1:50	(200ng/ml)
1:100	(100ng/ml)
1:200	(50ng/ml)
1:400	(25ng/ml)
1:800	(12.5ng/ml)
1:2000*	(5ng/ml)
1:8,000*	(1.25ng/ml)
1:16,000*	(0.625ng/ml)

*almost certainly requires the use of tyramide signal amplification (TSA).

Calculating the optimal hybridization temperature for your specific probe with in situ hybridization.

You can use the following formula to calculate the optimal temperature to hybridize your probe. The default temperature of 37°C is suitable for most of our probes. If you use a commercially available hybridization buffer, please follow the manufacturers specific instructions.

Calculating the T_{hyb} for GeneDetect® oligonucleotide probes for *in situ hybridization*

These calculations assume that you use our hybridization buffer recipe which has final concentrations of 4XSSC and 50% formamide.

Temperature for hybridization (T_{hyb} recommended, °C) = 24.21 + 0.41(%GC) - 500/n

n=length of probe

%GC (percentage of probe that is G or C)

If your probe/target hybrid has mismatches you should reduce the hybridization temperature by 1.4°C for each 1% of mismatches.

Example 1: For a probe with a total length of 48 base pairs, where 30 base pairs are either G or C.

T_{hyb} optimal = 24.21 + 0.41(62.5) - 500/48
T_{hyb} optimal = 24.21 + 25.62 – 10.41
T_{hyb} optimal = 39.42°C

Example 2: **For the control Poly(dT) probe** with a total length of 48 base pairs, where %GC = 0.

T_{hyb} optimal = 24.21 + 0.41(0) - 500/48
T_{hyb} optimal = 24.21 – 10.41
T_{hyb} optimal = 13.8°C

Hybridization of the control probe at room temperature will normally suffice.

Hybridize the control polydT probe at RT or lower. DO NOT add polyA to your hybridization buffer when using the control polydT probe.

Washes - Stringency and temperature.

General rules:

With in situ hybridization it is better to use LOW stringency hybridization conditions followed by HIGH stringency washing to remove non-specific bound probe rather than trying to reduce non-specific binding with high stringency hybridization.

Reduction of the salt concentration in the wash buffers to 1X and 0.5XSSC (from 4XSSC used in the hybridization buffer) increases the stringency of the washes.

Washing temperatures used should be approximately 5-20°C above the T_{hyb}

Thus for a probe hybridized at 37°C, washing at 55°C is at the upper stringency range.

Thus for the polydT probe hybridized at T_{hyb} optimal = 13.8°C washing should be performed at 18.8-33.8°C (NOT 55°C).

Notes and definitions:

Tm – the temperature at which 50% of an oligonucleotide probe and its perfect complement are in duplex. The Tm reduces by 1.4°C for each 1% of mismatches.

The standard calculations for determining Tm (also known as the melting temperature of an oligonucleotide are):

A. For oligonucleotides between 14-20 base pairs in length (Wallace rule)

Tm = 2 x (number of AT pairs) + 4 x (number of GC pairs)

B. For larger oligonucleotides (and taking into account Na^+ and formamide concentrations)

$Tm = 81.5 + 16.6(\log.Na^+) + 0.41(\%GC) – 0.61(\%\ formamide) – (500/n)$

Na+ = concentration of Na^+ ions in moles per liter in the hybridization buffer

4XSSC, Na^+ ion con = 780mM
1XSSC, Na^+ ion con = 195mM
0.5XSSC, Na^+ ion con = 97.5mM

n = length of the probe
% formamide = % of formamide in the hybridization buffer

How to calculate hybridization temperature from the Tm?

Classically T_{hyb} = Tm – 25 (for DNA:DNA hybrids of length ~100mer)

The optimal T_{hyb} for DNA:RNA hybrids on the other hand (i.e. for in situ hybridization or northern blotting with oligonucleotide probes to detect RNA) is suggested as being 10°C higher.

i.e. T_{hyb} = Tm – 15 (for DNA:RNA hybrids of length ~ 40-50)

However, we prefer to ensure 100% hybridization of the probe by using lower hybridization temperatures as suggested in the classic equation but ensure signal specificity with stringent washing.

One way to reduce background staining is to examine the effect of raising the hybridization temperature incrementally in 5°C steps. Alternatively, keep the hybridization temperature constant and test the effect of incrementally increasing the wash temperature in 5°C steps.

Confirming probe concentration.

Add an aliquot of the resuspended probe to a final volume of 1ml water (1000μl). Vortex or pipette up and down for 15 seconds. Read the absorbance of this dilution at A260nm. Use the formula below to calculate the concentration of the oligonucleotide probe (in nmol/μl).

Beer's Law: A (x dilution factor) = E x L x C

where:

A is the absorbance at 260nm (adjusted by the dilution factor)
E is the extinction coefficient, which is approximated to be 10μl/(nmol·cm) per nucleotide in the oligonucleotide
L is the path length of the cuvette used, typically 1cm
C is the concentration of the solution in nmol/μl

For example, the A260 reading for a 1:500 dilution of a 25mer stock was 0.50. To determine the concentration of the solution, rearrange Beer's Law to:

C = (A x dilution factor)/E x L= (0.50 x 500) / (10 x 25 bps) x (1) = 1.0nmol/μl

Dilution factor = 1000/2 = 500, if you added 2μl probe to 1000μl water.

All scientific enquiries to Scientific@GeneDetect.com
We WILL respond within 24 hours.

Updated June 20, 2006.