DIG Quick Guide

Important Information at a Glance

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DIG Labeling Techniques: Template Amount and Probe Sensitivity

Method	Template Amount	Probe Sensitivity
PCR Labeling	• 10 pg Plasmid DNA • 10 ng Genomic DNA	0.1-0.03 pg DNA
Random Prime Labeling	• 1 µg Plasmid DNA	0.1-0.03 pg DNA
High Prime Labeling	• 300 ng Plasmid DNA	0.1-0.03 pg DNA
In Vitro Transcription	• 1 µg Linearized Plasmid	0.01-0.03 pg DNA or RNA
3‘ End Labeling: Oligonucleotides	• 100 pmol Oligonucleotide	10 pg DNA or RNA
3‘ Tailing: Oligonucleotides	• 100 pmol Oligonucleotide	1 pg DNA or RNA

TIP

PCR labeling is a simple procedure for producing DNA probes, and is a good alternative to “conventional” random prime labeling.

Random Prime Labeling and High Prime Labeling: Typical Yield

Method	Yield after 1 Hours	Yield after 20 Hours
Random Prime: 1 µg	260 ng	780 ng
High Prime: 300 ng	450 ng	2000 ng
High Prime: 1 µg	850 ng	2300 ng

TIP

High Prime is an optimized system for random prime labeling. An overnight incubation leads to significantly higher yields with both procedures.

Amount of Starting Template Recommended for Southern and Northern Blots

Blot Types	Type of Probes	Amount of Starting Template Used in Blots
Southern Blots	DNA or RNA	• Plasmid DNA: < 1 ng • Genomic DNA: 1 – 5 µg
Northern Blots	RNA	• Total RNA: 1 µg • mRNA: 100 ng
	DNA	• Total RNA: 5 µg • mRNA: 500 ng

TIP

Use higher amounts of DNA (up to 10 µg)only in Southern Blots of genomic DNA with complex genomes. More than 10 µg of genomic DNA should not be used.

Recommendation for Probe Concentration during Hybridization

Type of Probe	Concentration
Random Prime or High Prime Labeled DNA	25 ng/ml
PCR Labeled DNA	2 µl/ml
RNA Probe Per *In Vitro* Transcription	50-100 ng/ml
3‘ End Labeled Oligonucleotides	1-10 pmol/ml
3‘ Tailed Oligonucleotides	0.1-10 pmol/ml

TIP

The optimal probe concentration is important for the hybridization result: an amount that is too small does not result in the desired sensitivity. A concentration that is too high leads to background.

Hybridization Temperatures with DIG Easy Hyb

Type of Hybrids	Temperatures
DNA : DNA	37-42°C
DNA : RNA	50°C
RNA : RNA	68°C

TIP

DIG Easy Hyb delivers optimal results in the hybridization. Other hybridization buffers with 50% formamide can be used at the same temperature. Northern blots should not be done in formamide-free buffers.

Stringent Blot Washes: Low Stringency

Type of Hybrids	Buffer	Period	Temperature
All	2xSSC, 0.1% SDS	2 x 5 min	Room Temperature

Stringent Blot Washes: High Stringency

Type of Hybrids	Buffer	Period	Temperature
DNA:DNA	0.5xSSC, 0.1% SDS	2 x 15 min	65°C
RNA:DNA or RNA:RNA	0.1xSSC, 0.1% SDS	2 x 15 min	68°C

TIP

The high-stringency washing steps should not be lengthened.

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