



Manual for Duplex Lectin-35S

I. Description

The Duplex Lectin-35S is a PCR System for the LightCycler to quantify Lectin (soya) and 35S promoter (GMO) in the same capillary.

The following specifications can be achieved:

Detection limit: 0.02%

Working range for the quantification above 0.1%

Variation of results: 12% (relative to the standard)

Uncertainty: +2.8% –1.6%

II. Product Components

Material enclosed by the Kit :

■ **MasterMix Lectin-35S** (blue tube)

■ **Hybprobes Lectin** (violet tube)

■ **Hybprobes 35S** (red tube)

■ **5% RR-Standard** (white tube), Standard Panel (Roundup Ready soya, certified from Fluka)

Storage Conditions:

+4°C at least 6 months, **+4°C** after solving in water 1 month

Never freeze the components, except the 5% RR Standard Panel

Hybprobes avoid light exposition

To **avoid contamination** store GMO-Standard (RR) separated from the other components!

Material NOT enclosed by the Kit :

LightCycler-**FastStart** DNA Master Hybridization Probes (Taq Polymerase)

Water (PCR-grade, for example from Roche Kit) = sH₂O

Tubes (RNase, DNase free)

LightCycler instrument (Roche)

III. Additional Components

Available for free on request, delivered as attachment (e-mail) and/or diskettes

■ **Setup Sheet:** EXCEL file for PC and Macintosh

■ **Calculation Sheet:** EXCEL file for PC and Macintosh

■ **Experiment File:** PCR 11 250700 (thermal cycling program to be used with the LightCycler Program. Copy the files into the Protocol folder of the LightCycler Programme before use)

■ **Sample Data Entry File:** Duplex L-Z-35S.SAM (to be used with the LightCycler Program. Copy the files into the Protocol folder of the Lightcycler Program before use)

IV. Analysing Strategy

Use different solutions for the template (sample DNA). For example 3 dilutions: 160ng, 80ng, 40ng of template DNA (photometrically determined) per assay.

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V. Ordering information

Product	Size	Cat.#
Duplex Lectin-35S The Duplex Lectin-35S is a PCR System for the LightCycler to quantify Lectin (soya) and 35S promoter (GMO) in the same capillary. For Research Use Only. Not for use in diagnostic procedures.	96 Assays	22003

related Products	Size	Cat.#
Duplex Zein-35S The Duplex Zein-35S is a PCR System for the LightCycler to quantify Zein (maize) and 35S promoter (GMO) in the same capillary. For Research Use Only. Not for use in diagnostic procedures.	96 Assays	22004
Allin 1.0 Qualitative Multiplex Screening System for Zein (maize), Lectin (soya) and 35S promoter (GMO). Including inhibition Control and positive Control. For Research Use Only. Not for use in diagnostic procedures.	3x20 Assays 6x20 Assays	Promega Promega

VI. Reaction Protocol

Preperations

To solve each component add water (water PCR grade, for example from Roche Kit), mix it gently (do not vortex!). Hybprobes should be blue, Mastermix transparent. **Store the components at +4°C for one hour** for completely solving.

MasterMix (48x)	dissolve with	383 µl sH2O
Hybprobes Lectin (48x)	dissolve with	12 µl sH2O
Hybprobes 35S (48x)	dissolve with	12 µl sH2O

RR Standard-Panel:

take 4 tubes (RNase DNase free). Write 160ng/10µl, 64ng/10µl 32ng/10µl, 16ng/10µl, 3.2ng/10µl
Solve original 5% RR-Standard in 120µl water, vortex it briefly

Dilutions	from previous Dilution	water	total for at least 4 panels (10µl/per assay)
RR 160 ng/10µl	100		50
RR 64 ng/10µl	50	75	75
RR 32 ng/10µl	50	50	50
RR 16 ng/10µl	50	50	80
RR 3.2 ng/10µl	20	80	100

storage recommendation:

Make aliquots (for example 12µl) and freeze it at **-20°C**. Do not refreeze it. Store Panel at 4°C for max 4 days.

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Amplification Reactions (use **Setup Sheet**, see Chapter III.)

Component	Single Reactions	12 Reactions (+5%)
MasterMix Lectin-35S	7.6µl	95.76µl
Hybprobes Lectin	0.2µl	2.52µl
Hybprobes 35S	0.2µl	2.52µl
LightCycler-FastStart DNA Master Hybridization Probes	2µl	25.2µl
Mix gently (no vortex!)		
Distribute to each capillary	10µl	10µl
add sample DNA (160ng/10µl) or standard	10µl	10µl
Total per capillary	20µl	20µl
Seal each capillary with a stopper centrifugate at 700 x g for 5 s (3000rpm in a standard benchtop microcentrifuge)		

Thermal Cycling Programme (use **Experiment File**: PCR 11 250700, see III.)

Ramp rate 20°C/sec
Gain F1: 1 F2: 50 F3: 100

Denat	Time	Temperature
	10min	95°C

Cycling 45x	Time	Temperature	Acquisition mode
	95°C	10sec	
	60°C	10sec	single
	72°C	10sec	

Melting curve	Time	Temperature	Acquisition mode
	95°C	10sec	
	55°C	10sec	
	95°C	0.2°C/sec ramp rate	Continuous

Cooling	Time	Temperature
	10sec	40°C

Edit samples: use **Sample Data Entry File**, see Chapter III

Data interpretation

Chanel F2: 35S-signal; F3: Lectin-signal
 Baseline proportional
 Points above crossing line 2
 Position of the noise band manually adjusted (where Mean Squared Error is minimal (r=1))
Fill in the the Calculation Sheet (see Cahpter III.) with data from the LightCycler. Use results which behave approximately proportional to the dilution of the sample.

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 Berne, 9 August 2001