

DELFI[®] hCG Reagents R007-101

For Scientific Research Use Only.

This product is not to be used for *In Vitro* or *In Vivo* Diagnosis.

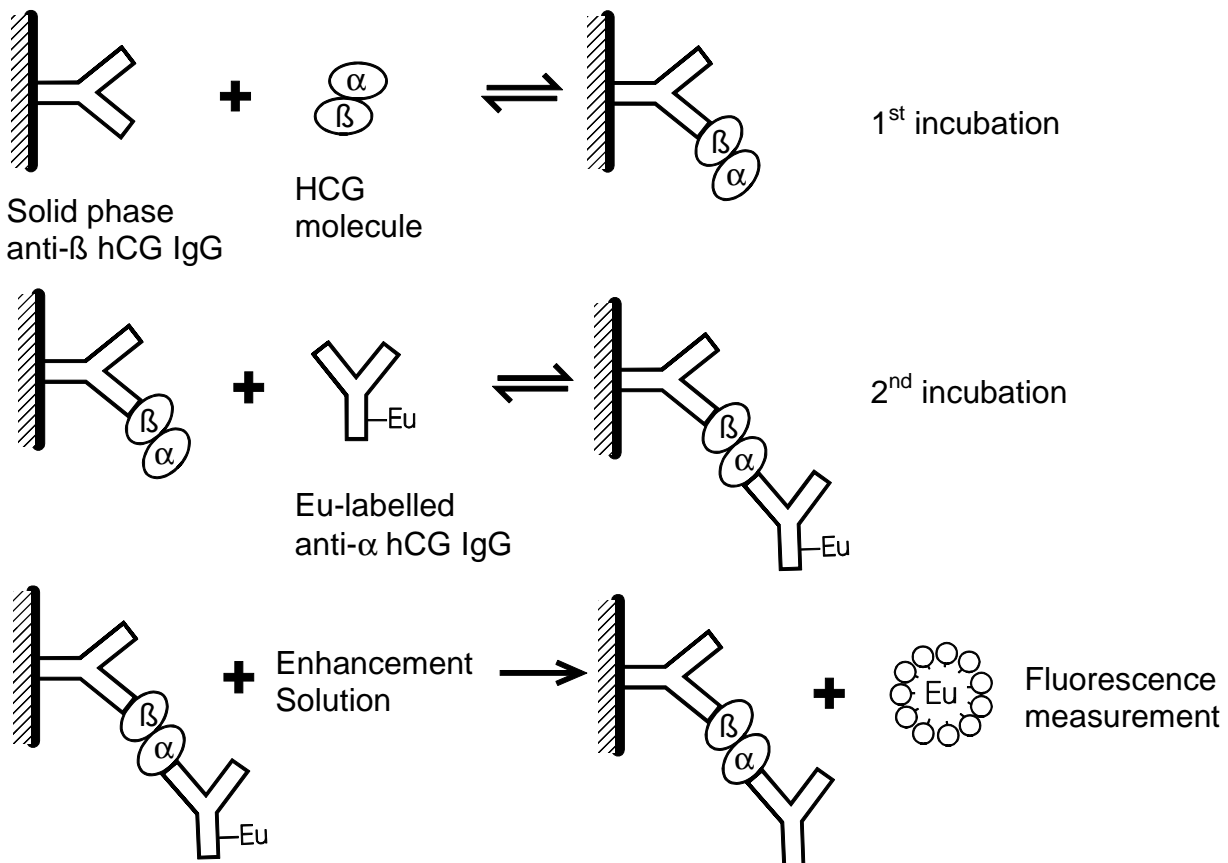
PRINCIPLES OF THE ASSAY

This product has been developed for the quantitative determination of human chorionic gonadotropin (hCG) in serum.

The DELFIA[®] hCG assay is a solid phase, two-site fluoroimmunoassay in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the hCG molecule. Standards, controls and samples containing hCG are first reacted with immobilized monoclonal antibodies directed against a specific antigenic site on the β subunit of hCG. Europium-labelled antibodies directed against a specific antigenic site on the α subunit are then reacted with the intact hCG already bound to the solid-phase antibody.

Enhancement Solution dissociates europium ions from the labelled antibody into solution here they form highly fluorescent chelates with components of the Enhancement Solution. The fluorescence is proportional to the quantity of hCG in the sample (1,2).

It is also possible to run a rapid assay using only two standards and shorter incubation times.



PACKAGE CONTENTS

Each DELFIA[®] hCG package contains reagents for 96 assays.

The expiry date of the complete package is stated on the outer label. Store at +2 - +8°C.

Reagents

Component	Quantity	Shelf life and storage
hCG Standards (approx. values)	7 vials, 1.0 mL	+2 - +8°C until expiry date stated on the vial label.
A 0 U/L	The exact hCG concentrations are given on the lot specific quality control certificate included in the package.	
B 2 U/L		
C 10 U/L		
D 100 U/L		
E 1000 U/L		
F 5000 U/L		
G 10000 U/L		

The ready-for-use standards are in normal human male serum with < 0.1 % sodium azide as preservative.

The standards have been calibrated against the WHO 3rd International Standard of human chorionic gonadotropin for immunoassay (coded 75/537).

Anti-hCG-Eu tracer stock solution (~ 20 µg/mL) (mouse monoclonal)	1 vial, 0.95 mL	+2 - +8°C until expiry date stated on the vial label.
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The tracer is in Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, and < 0.1 % sodium azide as preservative.

Wash Concentrate	1 bottle, 40 mL	+2 - +8°C until expiry date stated on the bottle label.
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A 25-fold concentration of Tris-HCl buffered (pH 7.8) salt solution with Tween 20. Germall II¹ added as preservative.

DELFIA Buffer	1 bottle, 50 mL	+2 - +8°C until expiry date stated on the bottle label.
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Ready-for-use Tris-HCl buffered (pH 7.8) salt solution with bovine serum albumin, bovine globulin, mouse IgG, Tween 40, an inert red dye, and < 0.1 % sodium azide as preservative.

¹ Germall is a registered trademark of Sutton Laboratories Inc.

Enhancement Solution	1 bottle, 50 mL	+2 - +8°C until expiry date stated on the bottle label. Shelf life 6 months at room temperature (+20 - +25°C). Avoid direct sunlight.
Ready-for-use Enhancement Solution with Triton X-100 ² , acetic acid and chelators.		
Anti-hCG Microtitration Strips. 8 x 12 wells coated with antibodies against the β subunit of the hCG molecule (mouse monoclonal)	1 plate	+2 - +8°C until expiry date stated on the label. Make sure that the plastic pack remains sealed.
Lot specific quality control certificate	1 pc	

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE PACKAGE

The DELFIA hCG reagents are part of a complete system of reagents and instrumentation. The DELFIA system requires the following items, which are available from PerkinElmer Life Sciences or its distributors.

1. Time-resolved fluorometer plus printer and (optional) computer
2. Automatic washer - DELFIA Platewash (prod. no. 1296-026)
3. Automatic shaker - DELFIA Plateshake (prod. no. 1296-001/002 or 1296-003/004)
4. Pipette for dispensing DELFIA Buffer and the diluted tracer solution - Eppendorf Multipipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively DELFIA Plate Dispense with the DELFIA Dispense Unit (prod. nos. 1296-041 and 1296-043)
5. Pipette for dispensing the Enhancement Solution - Eppendorf Multipipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively the DELFIA Plate Dispense (prod. no. 1296-041)
6. DELFIA Diluent I (prod. nos. B127-100 and B128-100), DELFIA Diluent II (prod. nos. B131-100 and B132-100) or DELFIA Assay Buffer (prod. nos. 1244-106 and 1244-111)

In addition to the DELFIA system the following are required:

- precision pipettes for dispensing microlitre volumes
- pipettes for dispensing the millilitre volumes of buffer required to prepare the tracer dilution
- distilled water

² Triton is a registered trademark of Rohm and Haas Co.

COLLECTION AND HANDLING OF SERUM AND PLASMA SAMPLES

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Plasma samples can also be used. Haemolytic, lipaemic or icteric samples do not interfere with the assay.

If the hCG concentrations in samples exceed (or are expected to exceed) 5000 U/L, they should be diluted 100-fold with DELFIA Diluent I, Diluent II or Assay Buffer to bring them below 5000 U/L.

Samples can be stored 6 days at +2 - +8°C. For longer periods store samples at -20°C. Repeated freezing and thawing should be avoided to prevent hCG denaturation.

WARNINGS AND PRECAUTIONS

For scientific research use only. This product is not to be used for *in vitro* or *in vivo* diagnosis.

This package contains reagents manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies and found to be negative. Nevertheless all recommended precautions for the handling of blood derivative should be observed. Please refer to the U.S. Department of Health and Human Services publication "Biosafety in Microbiological and Biomedical Laboratories" or any other local or national regulation.

Reagents contain sodium azide (NaN₃) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Disposal of all waste should be in accordance with local regulations.

ASSAY PROCEDURE

Perform each determination in duplicate for both standards and unknowns. A standard curve should be run with each assay. All reagents and samples must be brought to room temperature (+20 - +25°C) before use.

1. Preparation of reagents

Reconstituted stability

Wash solution

2 weeks at +2 - +25°C in a sealed container.

Pour the 40 mL of Wash Concentrate into a clean container and dilute 25-fold by adding 960 mL of distilled water to give a buffered wash solution (pH 7.8).

Anti-hCG-Eu tracer

Prepare within one hour of use.

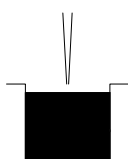
Prepare the needed volume of tracer dilution by mixing 60 μL tracer stock solution with 3.0 mL of DELFIA Buffer per strip (see table on the Summary Protocol Sheet).

It is important that the DELFIA Buffer does not come into contact with tracer stock solution not intended for immediate use.

We advise the use of a disposable plastic container to prepare the tracer working solution.

- Transfer the required number of microtitration strips to a strip frame. (Return the remaining strips to the plastic tray pack and reseal.) Do not take more strips than can be easily handled within 30 minutes.
- Pipette 25 μL of duplicates of the hCG Standards (Std) and samples (unknowns - Unk) into the strip wells according to the following table.

1	2	3	4	5	6	7	8	9	10	11	12	Strip
Std A	Std A	Std B	Std B	Std C	Std C	Std D	Std D	Std E	Std E	Std F	Std F	A
Std G	Std G	1st Unk	1st Unk	2nd Unk	2nd Unk	3rd Unk	3rd Unk	etc.				B
												C etc.

- Add 200 μL of DELFIA Buffer to each well using **the recommended Eppendorf Multipipette** after discarding the first aliquot, or use the DELFIA Dispense Unit. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or the surface of the liquid. 
- Incubate the frame for 1 hour (\pm 10 minutes) at room temperature with **slow** shaking using the DELFIA Plateshake.
- After the incubation step, aspirate and wash each strip with the DELFIA Platewash using program 7 (wash 1).
- Add 200 μL of diluted Anti-hCG-Eu tracer solution to each well. Pipetting should be as for the DELFIA Buffer in step 4 above.
- Incubate the frame for 30 minutes (\pm 10 minutes) with **slow** shaking at room temperature.

9. After the second incubation step, aspirate and wash each strip with the DELFIA Platewash using program 7 (wash 2).
10. Add 200 µL of Enhancement Solution directly from the reagent bottle to each well using **the recommended Eppendorf Multipette** after flushing the Combitip once with Enhancement Solution (to waste), or use the DELFIA Plate Dispense. Refill the Combitip and discard the first aliquot. Avoid touching the edge of the well or its contents.
11. Shake the frame **slowly** for 5 minutes. The fluorescence is stable for several hours if evaporation is prevented. However, we recommend measurement within 1 hour as external factors may cause a decrease in signal with time, although this is extremely rare.
12. Ensure that each strip is firmly seated in the frame and measure the fluorescence in the time-resolved fluorometer.

When using the 1232 or 1234 fluorometer select program 7 or MultiCalc^{® 3} protocol "7 HCG" for automatic measurement and result calculation.

When using VICTOR² D start the measurement from the Start Wizard, select "HCG" from Protocols/Kits panel "Fertility" and define the number of plates and samples.

Check the parameter group for program 7 or the MultiCalc protocol "7 HCG", and correct it, if it differs from the following (see fluorometer manual or MultiCalc manual for editing the parameters):

ASSAY TYPE	:	IFMA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LOGARITHMIC	
Y-AXIS	:	LOGARITHMIC	
BLANKS	:	2	
STANDARDS	:	6	
STANDARD REPLICATES	:	2	
STANDARD CONC	:	B	(Make sure that the hCG standard concentrations correspond to those given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)
STANDARD CONC	:	C	
STANDARD CONC	:	D	
STANDARD CONC	:	E	
STANDARD CONC	:	F	
STANDARD CONC	:	G	
UNKNOWN REPLICATES	:	2	

Rapid assay procedure

When only a small number of samples need to be assayed a rapid assay protocol can be followed. Use only the 0 and 1000 U/L standards for the standard curve. Shorten the two incubation periods to 30 and 15 minutes, respectively. Otherwise the assay procedure is exactly as described above.

³ MultiCalc is a registered trademark of Wallac Oy.
VICTOR is a trademark of Wallac Oy.

In order that results of the rapid assay can be meaningfully compared, it is important to limit the number of patient samples so that pipetting can be done in 5 minutes or less for each pipetting cycle. This will keep variations in the reaction (resulting from incomplete immunoreactions due to the short incubation times) within acceptable limits.

Create a new parameter group for automatic measurement and result calculation as follows:

ASSAY TYPE	:	IFMA	
FITTING METHOD	:	SPLINE SMOOTHED	
X-AXIS	:	LINEAR	
Y-AXIS	:	LINEAR	
BLANKS	:	0	
STANDARDS	:	2	(Make sure that the hCG standard concentrations correspond to those given on the lot specific quality control certificate. If this is not the case, enter the new concentrations.)
STANDARD REPLICATES	:	2	
STANDARD CONC	:	A	
STANDARD CONC	:	E	
UNKNOWN REPLICATES	:	2	

hCG values of the rapid assay procedure (incubation time: 30 + 15 minutes) are equivalent to concentrations obtained with the normal assay procedure (incubation time: 1 h + 30 minutes).

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the product. The reagents supplied with this package are intended for use as an integral unit. Do not mix identical reagents from packages having different lot numbers. Do not use reagents after the expiry date printed on the package label.
2. Reagents should be allowed to reach room temperature (+20 - +25°C) prior to sample preparation. Frozen samples should be brought to room temperature slowly and gently mixed by hand. Do not vigorously vortex or mix samples.
3. When washing the strips, ensure that each well is filled up completely to the top edge as shown in the figure. After washing the strips, check that the wells are dry. If there is moisture left, invert the plate and tap firmly against absorbent paper.



For detailed information on the cleaning and maintenance of the washing device, please refer to the DELFIA Platewash manual.

4. The avoidance of europium contamination and resulting high fluorescent background demands high standard pipetting and washing techniques. Thus it is extremely important to use the pipettes supplied with the DELFIA system for the recommended purposes only.
5. The Enhancement Solution should be dispensed using only the recommended Eppendorf Multipipette after the Combitip has been first flushed with Enhancement Solution according to the Directions for Use. The same Combitip must not be used for

pipetting any other reagent. After use place the Eppendorf Multipette on the pipette stand, with the Combitip still attached.

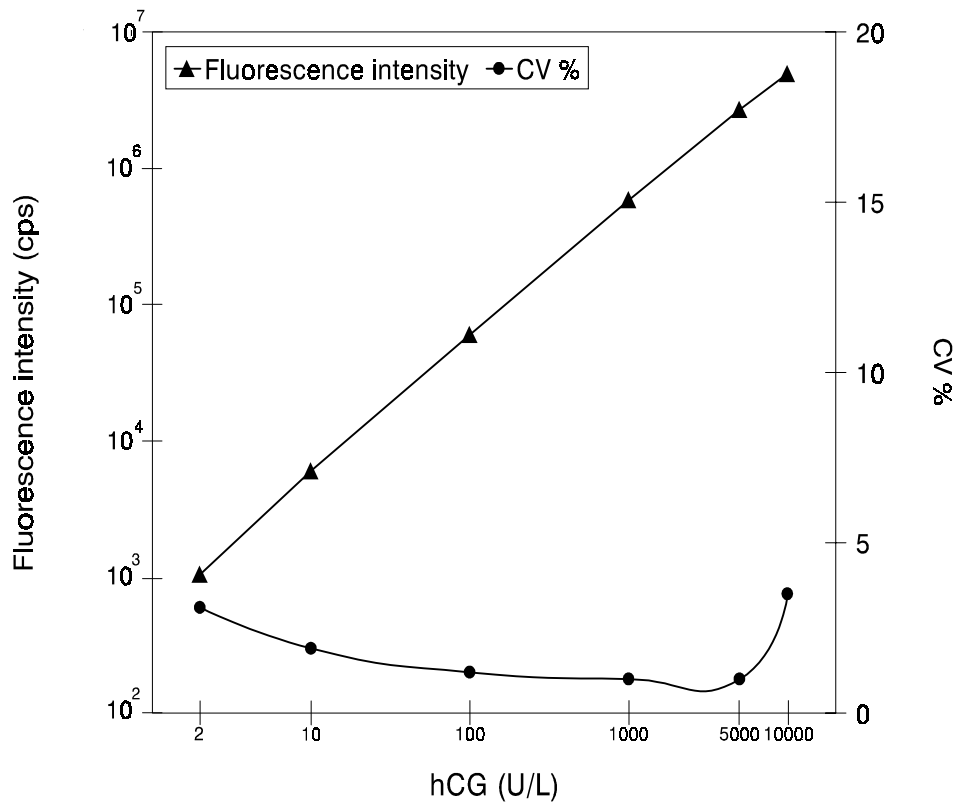
When using the DELFIA Plate Dispense and DELFIA Dispense Unit, please refer to the manual.

CALCULATION OF RESULTS

The DELFIA system incorporates programs for data reduction, and the results are obtained as printouts of standard curves, unknown concentrations etc. (see Fluorometer instrument manual or MultiCalc manual for detailed information).

ANALYTICAL PERFORMANCE CHARACTERISTICS

A typical standard curve and precision profile obtained with the DELFIA hCG assay are shown below. The precision profile was calculated from 308 duplicate measurements of serum samples using the MultiCalc data management program.



Precision⁴: The variation of the DELFIA hCG assay was determined in 27 runs with 3 replicates, and the analysis of variance approach was used to calculate the following variations:

Serum sample	Total mean value (U/L)	Intra-assay variation (% CV)	Inter-assay variation (% CV)	Total variation (% CV)
1	9.70	4.1	4.6	6.2
2	72.8	4.1	3.2	5.2
3	976	2.0	3.2	3.8

Analytical sensitivity⁵: The analytical sensitivity of the DELFIA hCG assay is typically better than 0.5 U/L if defined as the value which is 2 standard deviations above the mean of the zero standard measurement values (mean value + 2 SD) (n = 20).

Recovery⁶: Spiked serum samples were prepared by adding varying levels of concentrated hCG solution to pooled serum samples containing a known amount of hCG. Recoveries were in the range of 87 % to 112 %, with a mean value of 99.8 ± 7.1 % (SD) (n = 8).

Correlation⁷:

Rapid vs. normal assay:

The DELFIA hCG rapid assay (y) was compared with the DELFIA hCG normal assay (x) using samples in the range of 1.4 - 880 U/L. The correlation was found to be:

$$y = 1.02x + 5.8; \quad r = 0.99 \quad (n = 45)$$

Cross reactivity⁸: The cross reactivity of the DELFIA hCG assay with other hormones is presented in the following table where:

$$\text{Cross reactivity \%} = \frac{\text{Signal resulting from 1 ng/mL competing hormone}}{\text{Signal resulting from 1 ng/mL hCG}} \times 100$$

⁴ Study performed at Wallac Oy, Turku, Finland.

⁵ as above

⁶ as above

⁷ as above

⁸ as above

Hormone	% cross reactivity
*hLH	< 0.5
hTSH	0.08
hFSH	0.2
+hCG α subunit	1.9
+hCG β subunit	2.4

* The cross reactivity of hLH was calculated from serum samples after a gel filtration procedure which separates the hLH and hCG fractions (3). Percentage cross reaction is expressed as:

$$\frac{\text{Apparent hCG (U/L 3}^{\text{rd}} \text{ IS)}}{\text{hLH (U/L 1}^{\text{st}} \text{ IRP)}} \times 100$$

+ The apparent cross reactivity of the α and β hCG subunit may be due to hCG contamination of the preparations used.

Hook effect⁹: hCG is a two step assay. There is no hook effect up to hCG concentration 10^6 U/L.

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event Wallac Oy and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

Wallac Oy, its affiliates and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

⁹ Study performed at Wallac Oy, Turku, Finland.

REFERENCES

1. Soini, E. and Kojola, H. (1983): Time-resolved fluorometer for lanthanide chelates - a new generation of nonisotopic immunoassays. *Clin. Chem.* **29**, 65-68.
2. Hemmilä, I., Dakubu, S., Mukkala, V-M., Siitari, H. and Lövgren, T. (1984): Europium as a label in time-resolved immunofluorometric assays. *Anal. Biochem.* **137**, 335-343.
3. Stenman, U-H., Alfthan, H., Ranta, T., Vartiainen, E., Jalkanen, J. and Seppälä, M. (1987): Serum levels of human chorionic gonadotropin in nonpregnant women and men modulated by gonadotropin-releasing hormone and sex steroids. *J. Endocrinol. & Metab.* **64**, 730-736.
4. Pettersson, K., Siitari, H., Hemmilä, I., Soini, E., Lövgren, T., Hänninen, V., Tanner, P. and Stenman U-H. (1983): Time-resolved fluoroimmunoassay of human choriogonadotrophin. *Clin. Chem.* **29**, 60-64.
5. Stenman, U-H., Myllynen, L., Alfthan, H. and Seppälä, M. (1983): Ultrarapid and highly sensitive time-resolved fluoroimmunoassay for chorionic gonadotropin. *Lancet* **2** (8351) 647-649.

PATENTS

This test system is covered by the following patents:

Europe (Austria, Belgium, Italy, Switzerland, Holland, UK, France): 0064484, 0139675

Federal Republic of Germany: P32722605-08, P3462252.7

Sweden: 8102753-4

USA: 4,565,790, 4,808,541

Last revision March 2001

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DELFIA[®] hCG Reagents

Summary Protocol Sheet

Add standards and unknowns		25 μ L																											
Add buffer		200 μ L																											
Incubate		1 h slow shaking at RT or 30 min. slow shaking at RT (rapid protocol)																											
Dilute tracer (see table)		<table border="1"> <thead> <tr> <th>Strips</th> <th>Tracer stock solution (μL)</th> <th>Buffer (mL)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>60</td> <td>3</td> </tr> <tr> <td>2</td> <td>120</td> <td>6</td> </tr> <tr> <td>3</td> <td>180</td> <td>9</td> </tr> <tr> <td>4</td> <td>240</td> <td>12</td> </tr> <tr> <td>5</td> <td>300</td> <td>15</td> </tr> <tr> <td>6</td> <td>360</td> <td>18</td> </tr> <tr> <td>7</td> <td>420</td> <td>21</td> </tr> <tr> <td>8</td> <td>480</td> <td>24</td> </tr> </tbody> </table>	Strips	Tracer stock solution (μ L)	Buffer (mL)	1	60	3	2	120	6	3	180	9	4	240	12	5	300	15	6	360	18	7	420	21	8	480	24
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Wash		Program 7 (x 2)																											
Add tracer dilution		200 μ L																											
Incubate		30 min. slow shaking at RT or 15 min. slow shaking at RT (rapid protocol)																											
Wash		Program 7 (x 6)																											
Enhance		200 μ L, 5 min. slow shaking																											
Count		KIT 7 (check concentrations from QC certificate)																											