PROTEIN DISAGGREGATION KITS

PROTOCOL

Part # 64DAGGRPET & 64DAGGRPEG

Test Size#: 100 SAMPLES (64DAGGRPET), 500 SAMPLES (64DAGGRPEG)

Revision: 01 March/2021 **Store at:** 2-8°C

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid disaggregation of several aggregated proteins, such as Amyloid beta 1-42, TDP-43, or alpha-synuclein. Aggregated proteins are produced by cells and can be disaggregated after lysis of the cell membrane, or directly after secretion in cell culture media. The level of disaggregated protein in cell lysate or in cell culture media can be detected using the appropriate immunodetection kit for the targeted protein.

The disaggregation procedure for samples enables proteins to be extracted from aggregates. The antigen is then detected in a sandwich assay format.

The principle is based on two successive steps of reagent additions, without any need for centrifugation. Disaggregation buffer A is added to the sample. The mixture is left for 15 min at room temperature, then Disaggregation buffer B is added. Part of the original sample is also treated separately in the same way, but with Control detection buffer C, to generate an appropriate control. After treatment, samples are ready to use with immunodetection kits.

The signals from samples containing protein aggregates (treated with Control buffer C) can be compared to those from the same samples treated with Disaggregation reagents A and B. The ratio obtained between disaggregated/ aggregated signals from the same original samples is proportional to the level of protein aggregation.

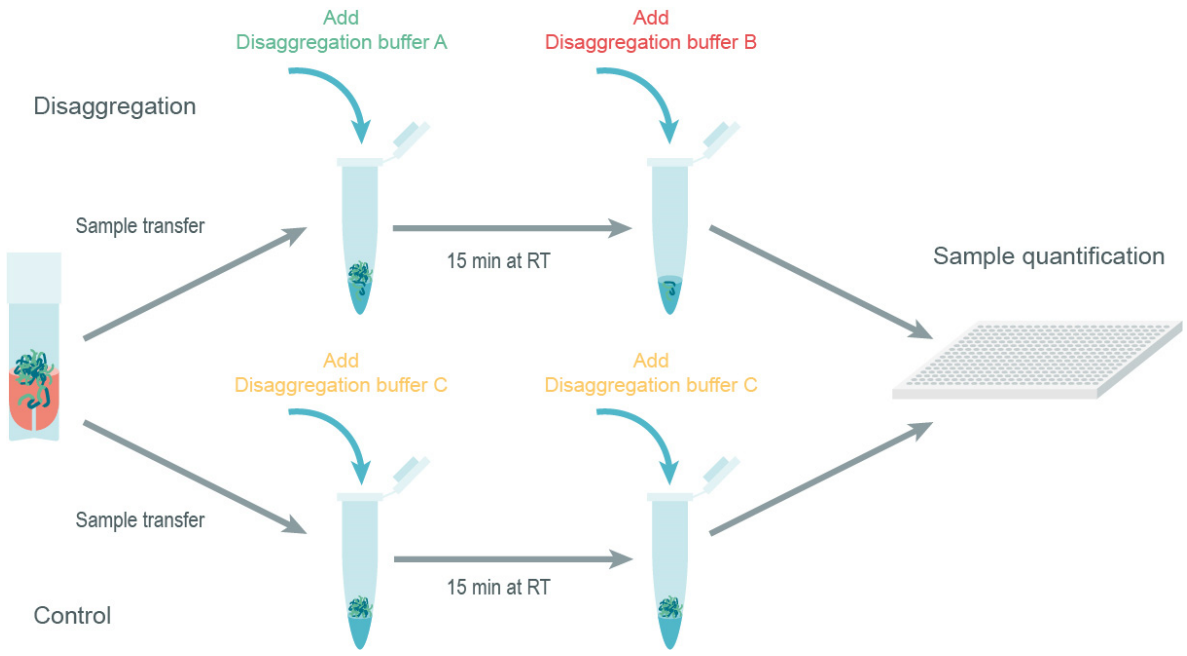

Figure 1: Principle of disaggregation step before immunodetection

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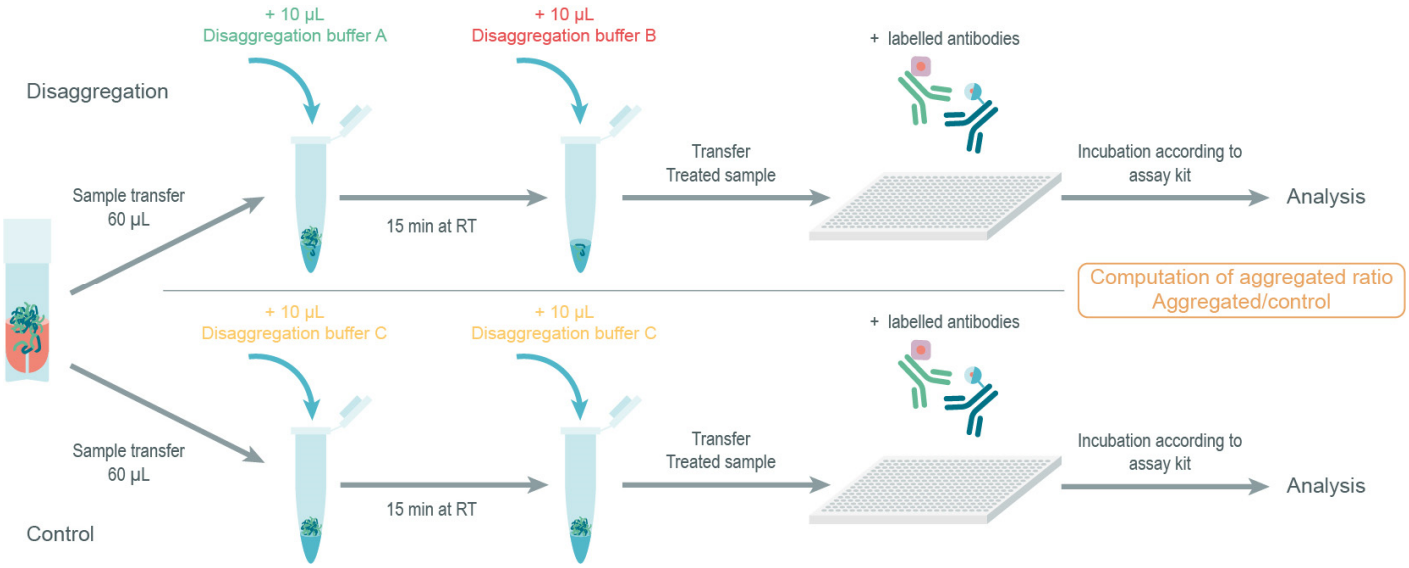
PROTOCOL AT A GLANCE



MATERIALS PROVIDED:

KIT COMPONENTS	STORAGE	100 SAMPLES CAT# 64DAGGRPET		500 SAMPLES CAT# 64DAGGRPEG	
Disaggregation buffer A (ready-to-use)	2-8°C	Green cap	1 vial – 1.8 mL	Green cap	3 vials – 1.8 mL
Disaggregation buffer B (ready-to-use)	2-8°C	Red cap	1 vial – 1.8 mL	Red cap	3 vials – 1.8 mL
Control detection buffer C (ready-to-use)	2-8°C	Yellow cap	2 vials – 1.8 mL	Yellow cap	6 vials – 1.8 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 100 and 500 samples respectively in 80 µL final.
Assay volumes can be adjusted **proportionally.**

STORAGE AND STABILITY

Store the kit at 2-8°C. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

SAMPLE PREPARATION

BEFORE YOU BEGIN:

- It is mandatory to collect and store samples in polypropylene microtubes, and then to treat samples in polypropylene microtubes (preferred) or polypropylene microplates.
- The efficacy of the disaggregation step can be checked by comparing the signal collected from the control sample (using Control detection buffer C) versus the disaggregated sample (using Disaggregation buffer A, then B).
- It is mandatory to keep the sample / buffer ratio (volume) constant for the disaggregation procedure (see below).
- We recommend testing several dilutions of samples (lysate or culture medium) to ensure that the samples are well disaggregated, and are within the assay linear range of the immunodetection assay.

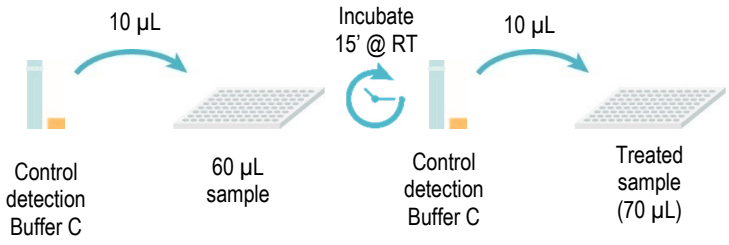
TO PREPARE SAMPLES:

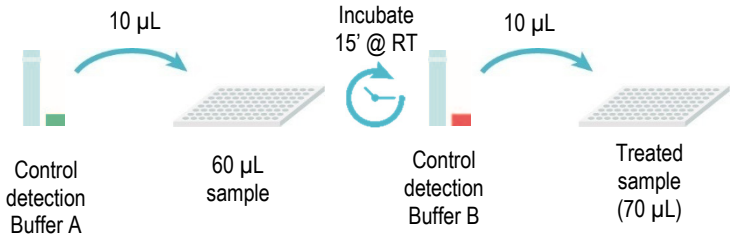
SAMPLE DISAGGREGATION PROCEDURE

This sample disaggregation protocol is optimized for microtubes.

Each sample is treated in two ways: control protocol or disaggregation protocol

- Transfer 6 volumes of sample (60 µL recommended) into a microtube
- Add 1 volume (10 µL recommended) of Disaggregation buffer A or Control detection buffer C.
- Vortex in microtubes (or by pipetting up and down at least 3 times, if using a microplate)
- Incubate for 15 minutes at room temperature.
- Add 1 volume (10 µL recommended) of the Disaggregation buffer B or Control detection buffer C.
- Vortex in microtubes (or by pipetting up and down at least 3 times, if using a microplate)

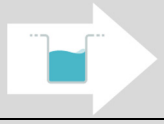
CONTROL PROTOCOL	
	First step: add Control detection buffer C to the sample (e.g. add 10 µL of Control detection buffer C to 60 µL of sample.) Incubation: 15' at room temperature. Second step: Add Control detection buffer C to the treated sample (e.g. add 10 µL of Control detection buffer C to 70 µL of treated sample.)

DISAGGREGATION PROTOCOL	
	First step: add Disaggregation buffer A to the sample (e.g. add 10 µL of Disaggregation buffer A to 60 µL of sample.) Incubation: 15' at room temperature. Second step: Add Disaggregation buffer B to the treated sample (e.g. add 10 µL of Disaggregation buffer B to 70 µL of treated sample.)

DISAGGREGATION ASSAY PROTOCOL

STANDARD PROTOCOL FOR DISAGGREGATION OR CONTROL DETECTION

		Sample	Disaggregation buffer A	Disaggregation buffer B	Control detection buffer C	Control detection buffer C
Sample	Disaggregation	60 µL	10 µL	10 µL		
	Control	60 µL	-		10 µL	10 µL

	SAMPLES	
Step 1 	Dispense 60 µL of each sample into a microtube (or a microplate)	
Step 2 	Add 10 µL of Control detection buffer C Mix and incubate 15 min at RT	Add 10 µL of Disaggregation buffer A Mix and incubate 15 min at RT
Step 3 	Add 10 µL of Control detection buffer C Mix	Add 10 µL of Disaggregation buffer B Mix
Step 4 	Test your sample with an assay kit for your targeted protein The Protein Disaggregation kit has already demonstrated full compatibility with several kits (Amyloid β1-40 ; Amyloid β1-42; TDP43 aggregation, α Synuclein total and, α Synuclein aggregation. For more information check our website)	

DATA INTERPRETATION

Calculate the Aggregated Ratio for each sample.

$$\text{Aggregated Ratio} = \frac{\text{Disaggregated Sample Signal}}{\text{Control Sample Signal}}$$

For illustrations about use of the aggregated ratio, please visit this webpage.

RESULTS

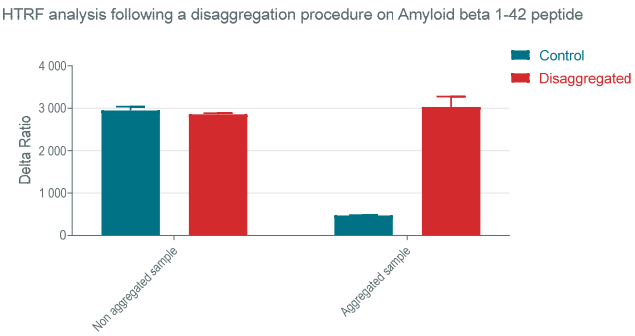
These data should be considered only as an example, using the HTRF Amyloid beta 1-42 assay (readings on HTRF compatible reader). Results may vary from one HTRF® compatible reader to another.

The data are drawn up by plotting the HTRF® Ratio of the sample treated as a control versus in a disaggregation condition, then calculating an Aggregated ratio per sample.

The signal linearity is dependent both on the cell line and on the total protein detected. A cell density optimization is highly recommended to ensure you work in optimal conditions.

Results are from a biochemical Human Amyloid beta 1-42 peptide aggregation protocol analysed with the Human Amyloid beta 1-42 HTRF assay, Cat # 62B42PEG. The same concentrations of highly aggregated and non-aggregated peptide were treated following the disaggregation procedure. 16 µL of control or disaggregated samples were transferred into a small volume detection plate to detect Human Amyloid beta 1-42, using the HTRF kit. In these test conditions, as expected, the highly aggregated peptide can only be detected after the disaggregation procedure (Fig. 1A). Computation of the aggregated ratio (Fig. 1B) enables a precise measurement of the aggregation level, and defines a unique value to report the protein aggregation.

1A.



1B.

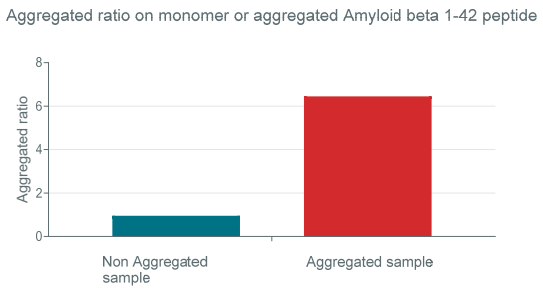


Figure 1A and 1B: HTRF analysis using the protein disaggregation kit followed by HTRF Human Amyloid beta 1-42 assay.
Data are from a collaboration with J. Torrent and V. Perrier, Institute of Neurosciences in Montpellier (INM), France.

	Control condition		Disaggregated condition		
Samples	Delta Ratio	CV %	Delta Ratio	CV %	Aggregated ratio
Non aggregated sample	2948	3	2858	1	0.96
Aggregated sample	470	2	3033	8	6.45