

Huntingtin (HTT) Quantitation Assays

FOR FURTHER INFORMATION:

Evotec SE
Essener Bogen 7
Hamburg, Germany
www.evotec.com

Dr Frank Herrmann
Principal Scientist,
HD Research Department
frank.herrmann@evotec.com

Dr Elizabeth van der Kam
Head HD Research Department
and VP Alliance Management
elizabethlouse.vanderkam@
evotec.com

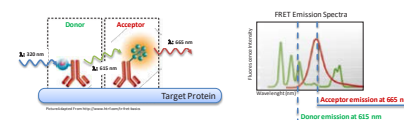
VALUABLE TOOLS TO SUPPORT THERAPEUTIC APPROACHES AIMED TO LOWER HTT EXPRESSION AND TO UNDERSTAND HTT PROTEIN CHANGES DURING DISEASE PROGRESSION IN PRE-CLINICAL AND CLINICAL SAMPLES

- ▶ Evotec offers services on a fee-for-service basis to address key issues in the Huntington disease (HD) research field.
- ▶ The portfolio includes versatile immunoassays for quantification of HTT protein (total and mutant soluble and aggregated HTT) in a range of tissues and biofluids of human, mouse and other species.

IMMUNOASSAY PLATFORMS

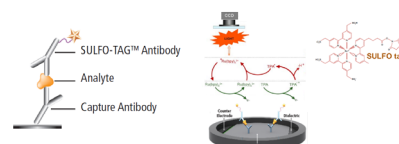
TR-FRET

- ▶ Ultra-high throughput
- ▶ Homogenous assay based on time-resolved fluorescence resonance energy transfer with simple SOP
- ▶ Conformation-specific



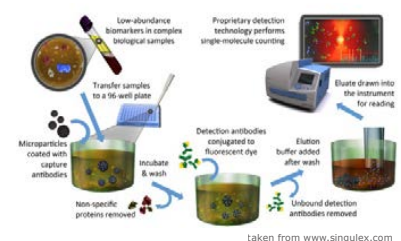
Meso-Scale Discovery (MSD)

- ▶ High throughput, high sensitivity (pM)
- ▶ Electrochemiluminescence detection assay, SULFO-TAG emits light upon electrochemical stimulation initiated at the electrode plate surface

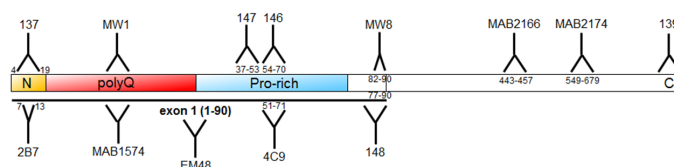


Single-Molecule Counting (SMC) – Millipore Erenna

- ▶ Ultra-high sensitivity (pM - fM) via magnetic particle-assisted confocal laser detection of single protein molecules.
- ▶ Extremely robust and reliable for clinical matrices such as complex human body fluids (plasma/CSF).



HTT ANTIBODIES USED IN IMMUNOASSAYS



HTT ASSAY MENU

	Assay	Assay target	Antibodies	Platform
mutant HTT	CHDI_HTT_005	Soluble mutant HTT protein	2B7-Tb / MW1-D2	TR-FRET
	CHDI_HTT_006	Soluble mutant HTT protein	2B7 / MW1	MSD
	CHDI_HTT_040	Soluble mutant HTT protein (ultrasensitive detection: recommended when biological material is limited, e.g. CSF samples)	2B7 / MW1	Singulex
aggregated HTT	CHDI_HTT_012	Smaller species of aggregated HTT	4C9-Tb / 4C9-Alexa	TR-FRET
	CHDI_HTT_013	Smaller species of aggregated HTT	MW8-Tb / MW8-D2	TR-FRET
	CHDI_HTT_045	Large species of aggregated HTT	MW8 / 4C9	MSD
HTT	CHDI_HTT_0039	Total soluble human HTT protein	2B7 / 4C9	MSD
	CHDI_HTT_003	Mouse HTT protein but not human HTT protein	CHDI-90000147 / MAB2166-4C8	MSD
	CHDI_HTT_119	Soluble mouse HTT (ultrasensitive detection)	CHDI-90000147 / MAB2166-4C8	Singulex
	CHDI_HTT_002b	Detection of HTT protein towards the N-terminus	CHDI-90000147 / MAB2166-4C8	MSD
	CHDI_HTT_004	Detection of HTT protein towards the C-terminus	CHDI-90000147 / MAB2166-4C8	MSD

CASE STUDIES

- ▶ WT mice with a neonatal injection of AAV into the lateral ventricle
- ▶ Brains sectioned and stained after 1 month. Images collected using 60x mag on Opera (4 channels, 11 planes, 0.5 μ m steps).
- ▶ Target mutant appears to act as a dominant negative
- ▶ Customized robust assay. Ability to run in 384-well format
- ▶ Successfully applied for analysis of total, mutant and aggregated HTT protein levels in HD-relevant cellular and rodent models to support pre-clinical HTT lowering studies.
- ▶ Quantification of HTT protein levels in diverse tissues (e.g. brain regions, liver, and muscle).

HTT quantification by MSD-based biomarker assays.

(A.) Detection of polyQ-expanded HTT in wild type, hetQ175 and homQ175 KI mouse whole brain lysates. (B.) Quantification of polyQ-expanded HTT and mouse HTT levels in HTT-shRNA-treated Q175 mice. (C.) Quantification of total and mutant HTT levels in a set of post-mortem HD patient and control donor brain tissues. (D.) Detection of aggregated HTT in the Q175 HD mouse model. (E.) Detection of aggregated HTT in the R6/2 HD mouse model.

