

# Adjuvant Activity of *Mycobacterium paratuberculosis* in Enhancing the Immunogenicity of Autoantigens During Experimental Autoimmune Encephalomyelitis

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## Materials

Name	Company	Catalog Number	Comments
anti-mouse CD115 antibody	Biologend, USA	135505	for cytofluorimetry 1:1,000
anti-mouse CD11b antibody	Biologend, USA	101215	for cytofluorimetry 1:1,000
anti-mouse CD11c antibody	Biologend, USA	117313	for cytofluorimetry 1:1,000
anti-mouse CD16/32 antibody	Biologend, USA	101302	for cytofluorimetry 1:1,000
anti-mouse CD4 antibody	Biologend, USA	116004	for cytofluorimetry 1:1,000
anti-mouse CD8a antibody	Biologend, USA	100753	for cytofluorimetry 1:1,000
anti-mouse I-A/I-E antibody	Biologend, USA	107635	for cytofluorimetry 1:1,000
anti-mouse Ly-6C antibody	Biologend, USA	128023	for cytofluorimetry 1:1,000
BBL Middlebrook OADC Enrichment	Thermo Fisher Scientific, USA	BD 211886	for isolation and cultivation of mycobacteria
C57BL/6J mice	Charles River Laboratory, Japan		3 weeks old, male and female
FBS 10279-106	Gibco Life Technologies, USA	42F9155K	for cell culture, warm at 37 °C before use
Freeze Dryer machine	Eyela, Tokyo, Japan	FDU-1200	for bacteria lyophilization
incomplete e Freund's adjuvant	Difco Laboratories, MD, USA	263810	for use in adjuvant
Middlebrook 7H9 Broth	Difco Laboratories, MD, USA	90003-876	help in the growth of Mycobacteria
Mycobacterium avium subsp. paratuberculosis K-10	ATCC, USA	BAA-968	bacteria from bovine origin
Mycobacterium tuberculosis H37 Ra, Desiccated	BD Biosciences, USA	743-26880-EA	for use in adjuvant
Mycobactin J	Allied Laboratory, MO, USA		growth promoter
Myelin Oligodendrocyte Glycolipid (MOG) 35-55	AnaSpec, USA	AS-60130-10	encephalotigenic peptide
Ovalbumin (257-264)	Sigma-Aldrich, USA	S7951-1MG	negative control antigen for proliferative assay

pertussis toxin solution	Fujifilm Wako, Osaka Japan	168-22471	From gram-negative bacteria <i>Bordetella pertussi</i> , increases blood-brain barrier permeability
Polytron homogenizer PT 3100	Kinematica		for mixing the antigen with the adjuvant
RPMI 1640 with L-glutamine	Gibco Life Technologies, USA	11875093	For cell culture
Thymidine, [Methyl- <sup>3</sup> H], in 2% ethanol, 1 mCi	PerkinElmer, Waltham, MA, USA	NET027W001MC	for proliferation assay, use (1 $\mu$ Ci/well)
Zombie NIR Fixable Viability Kit	Biolegend, USA	423105	cytofluorimetry, for cell viability